HOWARD HUGHES MEDICAL INC.
Lab Notebook #

Department Mol. Bio.
Subject 10/20/94 – 02/02/95.
Name ANN KIM #8.
Address

National Brand 43-648

Computation Notebook

Dennison Stationery Products Co., Framingham, MA 01701

75 Sheets
11¾" x 9¼"
4x4 Quad.

0 73333 43648 8

Ruben EXHIBIT #91

HTPB411 & HTPAN308 in PD10

Pg150 Book 7

10/20/94

Ligations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
HTPAN308 9/30	4	4	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10/20	—	—	—	6	6	6	—	—	—	—	—	—	—	—	—	—	—	—	—
HTPB411 9/30	—	—	—	—	—	—	9	9	9	—	—	—	—	—	—	—	—	—	—
10/20	—	—	—	—	—	—	—	—	—	6	6	6	—	—	—	—	—	—	—
PD10 1.1	1	—	—	—	—	—	—	—	—	—	—	—	6	6	6	—	—	—	—
PD1024 10/17	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	—
10/19	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—
10/19	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	11	11	11	9	9	9	12	12	12	9	9	9	9	9	9	15	15	15	17
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Incubate @ 16°C O/N

10/21/94

Add
Transform into M15 Chemically Competent Cells

Thaw M15 Cells on ice
Aliquot 100 µl into @ fresh Sterile tubes
Add 10 µl of Ligation Rx Tubes.
Incubate on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400 µl LB
Incubate 37°C 1 hr
Plate 175 µl onto LB + Amp + Kan plates
Incubate RT over the weekend

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75 Sheets
11¾" x 9¼"
4x4 Quad.

4 HTPB411 & HTPAN08 in PDI0

4/94

Plates look OK.

pPI0 1.1 + 2.4 looks very good so ligations worked well

PDI0 Vector colonies had many colonies
So did Vector + PDI0 fragment
Try PCR on colonies.

Pick 200 each into 200 ul LB+Amp+Kan in 96 well dish - HTPAN08 + HTPB411

Incubate 37°C 4 hrs w/aeration

PCR:

HTPAN08

		200x
10X PCR	3.2	640
10X dNTP	3.2	640
2499	0.2	40
2500	0.2	40
Taq	0.2	40
H_2O	23	4600
Cult.	2	

HTPB411

		200x
10X PCR	3.2	640
10X dNTP	3.2	640
2501	0.2	40
2502	0.2	40
Taq	0.2	40
H_2O	23	4600
Culture	2	

PCR Program #601.

95°C	5 min	
95°C	20 sec	30
55°C	20 sec	
72°C	1 min	
72°C	7 1/2 min.	
4°C	Hold.	

HTPB411 & HTPAV08 in PD10

10/24/94

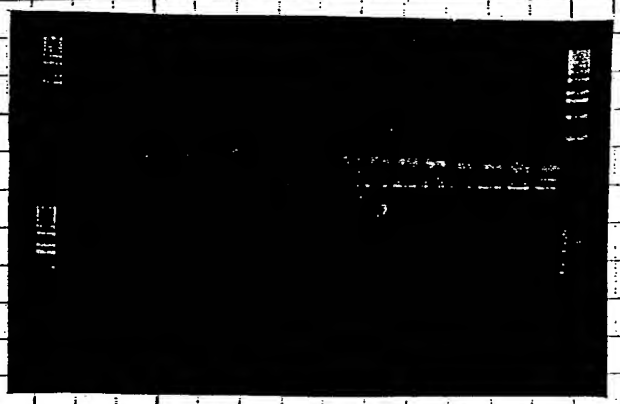
Run 10 µl on gel w/ 1 Kb ladder

- Nothing Worked!

Didn't Bother to take pictures.

HTPAV08

had strong size band.



Retry - Make New Primers today later.

- Phenol extract from PCR - then Ethanol ppt - Digest O/N.
- Run on LMP gel.

10/26/94

Try using 5' Bam primers from ligation into pAL Vector.

HTPAV08 - 7689

HTPB411 - 2429.

6 HTPANOB + HTPB4[1] in PDO

10/26/04 DNA long gel.

	HTPANOB ①	HTPANOB ②		HTPB4[1] ③	HTPB4[1] ④
10xPCR	10.0	10.0	10xPCR	10	10
10xdNTP	10.0	10.0	10xdNTP	10	10
2499	10.5	0.5	2501	0.5	0.5
2500	0.5	—	2507	0.5	—
7689	—	0.5	2429	—	0.5
Taq	0.3	0.3	Taq	0.3	0.3
H20	77.7	77.7	H20	77.7	77.7
DNA	1	1	DNA	1	1
	100	100		100 µl	100 µl

Run PCR Program #69. Modified

95°C 5 min
95°C 20 sec
55°C 20 sec
72°C 1 min
} 25X
72°C 7 1/2 min.
4°C Hold

Run 5 µl on gel with 1Kb ladder

looks good.

Add equal Volume PEG/NaCl
Sit on ice 10 min
Spin 10 min
Remove supernatant
1X 70% Ethanol wash. 500 µl
Spin 5 min
Remove supernatant.
Let pellet Dry Slightly
Resuspend in 20 µl 1X 0.1 TE
Add equal Volume Phenol
Vortex

HTPAD08 + HTPB[illegible] in RXO

10/26/94

Spin 5 min
Transfer upper Aqueous layer to fresh tube
repeat
Add 2 volumes Ethanol (400 µl)
1/10 volume 3M NaOAc. (20 µl)
Mix
Let sit on ice 1/2 hr
Spin 10 min
Remove supernatant
1X 70% Ethanol wash ~1000 µl
Spin 5 min
Remove supernatant
Allow pellet to dry
Resuspend in 200 µl TE
Set up digestions

DNA	100 µl
10X #2	20 µl
H_2O	79 µl
Xba/Bam	0.5/0.5
	200 µl

Incubate 37°C / overnight

10/27/94

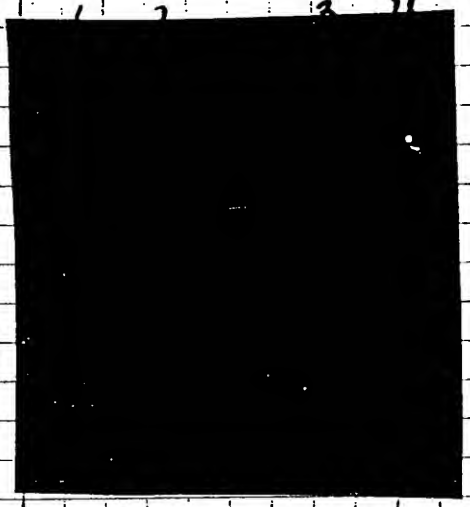
Run 10 µl on 1% gel with 1Kb ladder

Gel looks good
Add Sample Buffer and
Run on 0.8% LMP
Agarose gel
Run 80V 1/2 hrs.

Ethanol ppt Samples (see above) - 1X 70% Wash
Resuspend in 40 µl TE - Now Ready for gel

8 HTPAV08 & HTPB411 in PD10

10/27/94

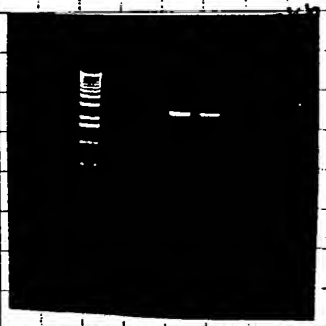


for Sample #1 cut out 1.8 Kb fragment too

Gene Clean
Add 1000 µl NaI
Heat 55°C 5 min
Spin well
Add 5ul Glass milk
Let incubate at Room temp 5 min w/ occasional mixing
Spin 7 sec.

3X [Remove Supernatant
Resuspend pellet in 400 µl Wash Buffer
Spin 7 sec
Remove Supernatant]
Spin 7 sec
Remove Supernatant
Resuspend pellet in 20 µl TE
Heat 55°C 1 min
Spin 7 sec
Transfer to fresh tube
Repeat 20 µl TE

Run 2 µl on gel with 1 Kb ladder



Use to set up ligations

DO NOT use the 1.3 Kb fragment from #1.

10/27/94

Ligation Reactions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
HTPAN08 #1	4	4	—	—	—	—	—	—	4	—	—	—	—	—	—	—
HTPAN08 #2	—	—	4	4	—	—	—	—	—	4	—	—	—	—	—	—
HTPB411 #3	—	—	—	—	4	4	—	—	—	—	4	—	—	—	—	—
HTPB411 #4	—	—	—	—	—	—	4	4	—	—	—	4	—	—	—	—
pD10 L1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4
pD10 10/19	1	—	1	—	1	—	1	—	—	—	—	—	1	—	—	1
pD10 10/12	—	1	—	1	—	1	—	1	—	—	—	—	—	1	—	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	—	—	—	—	—
H2O	12	12	12	12	12	12	12	12	13	13	13	13	16	16	17	12
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Incubate at 16°C O/N.

10/28/94

Transform MIS Chemically Competent cells.

Thaw cells on ice
Aliquot 100µl of cells into fresh tubes.
#16 may got 250µl.
Add 10µl of ligation to tubes
Incubate on ice 1 hr.
Heat 42°C 45 sec
Put on ice
Add 400µl LB
Incubate 37°C 1 hr
Plate onto LB + Amp + Kan plates. 100mm.
plates 1-16 100µl.
1-8 250µl also
plate #16 (100µl) mixed w/ #7.

10 HTPA008 & HTPB411 in PDID

10/28/94

Incubate 37°C O/N.
Asked Paul Moore to place at 4°C
Tomorrow.

10/31/94

Paul did not take plates out
- there were colonies. So try to PCR

PCR 2400 each 1-8 & PCR

	1+4 HTPA008				5+8 HTPB9			
	1-2	50	3-4	50	5+6		7+8	
10x PCR	3.2	160	3.2	160	3.2	160	3.2	160
10xdNTP	3.2	160	3.2	160	3.2	160	3.2	160
2499	0.2	10	0.2	10	2501 0.2	10	2501 0.2	10
2500	0.2	10	0.2	10	2502 0.2	10	2499 0.2	10
Taq	0.2	10	0.2	10	0.2	10	0.2	10
H2O	23	1150	23	1150	23	1150	23	1150
DNA	2	—	2	—	2		2	

PCR Prog 69.
Pithing set up.
Remake.
Try by pow.

PD 13

HTPB411 & HTPAN08 in PD10

Pg 12

10/31/94

Make more insert again
PCR w/ New and old primers.

	①	②	③	④
HTPAN08				
3'Xba 2499	0.2	—	0.2	—
3'Xba 2656	—	0.2	—	0.2
5'Bam 2500	0.2	—	—	0.2
5'Bam 7689	—	0.2	0.2	—
10X PCR	10	10	10	10
10X dNTP	10	10	10	10
Taq	0.2	0.2	0.2	0.2
DNA (10ng/μl)	1	1	1	1
H_2O	78.4	78.4	78.4	78.4
	100	100	100	100

	⑤	⑥	⑦	⑧
HTPB411				
3'Xba 2501	0.2	—	0.2	—
3'Xba 2653	—	0.2	—	0.2
5'Bam 2502	0.2	—	—	0.2
5'Bam 2429	—	0.2	0.2	—
10X PCR	10	10	10	10
10X dNTP	10	10	10	10
Taq	0.2	0.2	0.2	0.2
DNA (10ng/μl)	1	1	1	1
H_2O	78.4	78.4	78.4	78.4
	100	100	100	100

PCR Prog #619

95°C	5min	
95°C	20 sec	25X
55°C	20 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

14 HTPAN08 & HTPB411 in PD10

11/1/94

Ran 5ul on gel with 1Kb ladder.

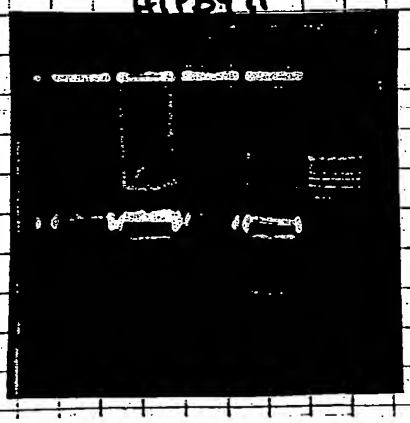
Add equal Volume PEG/NaCl
Spin 15 min
70% Ethanol Wash.
Resuspend 100 ul.
Digest O/N.

DNA	50 ul
H_2O	38
10x #2	10
Xba/Bam	1/1
	100 ul

Incubate reaction 3
37°C Overnight

11/2/94

Run Precipitate Digest
Add 1/10 Vol 3M NaAcetate
2 Vol 100% Ethanol
Spin 15 min
70% Ethanol Wash.
Allow pellet to dry
Resuspend in 45 ul of TE
Add 5 ul 10x Sample Buffer.
Run on 0.8% L.M.P. Gel.

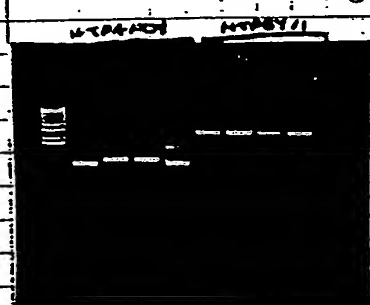


HTPAN08 + HTPBY11 & in pD10

11/2/94

Con
Cut out fragment
Gene clean
Add 1000 ul NaI
Heat ~~60~~ 65°C 2 min
Mix well
Add 7 ul Glass milk
Incubate at Room Temp 5 min
With occasional mixing
Spin 7 sec
Remove Supernatant
3X [Resuspend pellet in 400 ul Wash Buffer
Spin 7 sec
Remove Supernatant]
Spin 7 Sec
Remove Supernatant
~~Add~~ Resuspend pellet in 20 ul TE
Heat 65°C 1 min
Spin 10 sec
Transfer to fresh tube
Repeat with 20 ul TE

Run 2 ul of eluted fragment on 1% TAE gel with 1 Kb ladder



With fragments from 10/27 and the New Fragment Set up ligations

PEG/NaCl

in

gut

16 HTRANS08 HTRP411 into pD10

11/2/94

ligation Reactions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HTRANS08 2499+2500 4/22	3														
2499 + 7689		3													
11/2 2499+2500			3												
2656+7689				3											
2499 + 7689					3										
2656+2500						3									
HTRP411 2501+2502 4/27							3								
2501 + 2429								3							
11/2 2502+2502									3						
2653+2429										3	3				
2501 + 2429												3			
2653+2502															3
pD10 Bam/Hba	1	1	1	1	1	1	1	1	1	1	1	1	1	-	1
10X lig Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	13	13	13	13	13	13	13	13	13	13	13	13	14	17	13
T4 Ligase	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1
pD10 9.1kb															3

Incubate 16°C O/N.

11/3/94

Transform M15 cells
use 1-8 from 10/27 ligations
as well as 1-15 from 11/2

To 100 µl of cells add 10 µl of ligation
Let sit on ice 1 hr
Heat 42°C for 45 sec
Add 400 µl LB
Incubate 1 hr at 37°C
Plate onto 2 LB + Amp + Kan plates

HTPAN08 + HTPB411 in pD10

11/3/94

150 mm plates -
use 150 ul 1-8 & 1-12
use 300 ul 1-8 & 1-12

Incubate 37°C O/N.

11/4/94

Pick 1-8 10/27 [illegible]
into 200 ul LB + Kan + Amp plates
pick 1-12 11/2 into
200 ul LB + Amp + Kan

Incubate 37°C w/ aeration
Do PCR.
use internal primers to confirm

HTPB411 RP10 + FP15
HTPAN08 RP12 + FP18

HTPB411		200x	HTPAN08		200x
2400	0.25	50	1638	0.25	50
7945	1	200	7407	0.25	80
10X PCR	3.2	640	10X PCR	3.2	640
10X dNTP	3.2	640	10X dNTP	3.2	640
Taq	0.2	40	Taq	23.1	4620
H_2O	22.15	4430	H_2O	0.2	40
Culture	2		Culture	2	

PCR Program HG9

95°C 5 min
95°C 20 sec]
55°C 20 sec] 25x
72°C 1 min]
72°C 7 1/2 min
4°C hold

Store Ex at -20°C freezer labeled

(+) Control Plasmid
(-) Control H_2O

1
2
13
1
3

 HTPAN08 + HTPB411 in PDTO

11/7/94

Ran 10 µl of Reaction on gel with 1kb ladder

Nothing lit up again!

Talked w/ Steve -
Need to a remake primers,
Sites on old ones wrong!
Submitt both HTPAN + HTPB1
5' Bam a 3' ~~Ag~~ Xba.

11/8/94

3' Xba HTPB411 - Oligo Rec'd.
60°C O/N.

11/10/94

Rec'd ~~B~~ 5' Bam New HTPB411.
5' Bam New HTPAN08.

11/11/94

Rec'd 3' Xba New HTPAN08.

11/12/94

Dried Oligos
Ran PCR Reactions w/ New Primers

(PJ 39)

HTPAN08 / HTPB411 in PD10

pg 18

11/14/94

HTPAN08S04		HTPB411S15	
DNA (10ng/µl)	1	DNA (10ng/µl)	1
3'Xba Nest	0.6	3'Xba Nest	0.6
5'Bam Nest	6	5'Bam Nest	6.7
10X dNTP	10	10x dNTP	10
10X PCR	10	10X PCR	10
H_2O	72.1	H_2O	71.4
Taq	0.3	Taq	0.3
	100		100

PCR Program #69 modified

95°C	5min	
95°C	20sec	25X
55°C	20sec	
72°C	1min	
72°C	7½ min	
4°C	Hold	

HH Run 5µl on gel w/ HB Ladder

↑ 2nd Digest

Add equal Volume PEG/NaCl
Spin 10 min
Pour off Supernatant
Wash 1 ml 70% Ethanol
Spin 5 min
Rinse Supernatant
Resuspend in 100µl TE
Digestions

DNA	25
H_2O	19
10X #2	5
Xba/Bam	0.5/0.5
	50µl

Incubate RT ~~ok~~ fill Monday 11/14

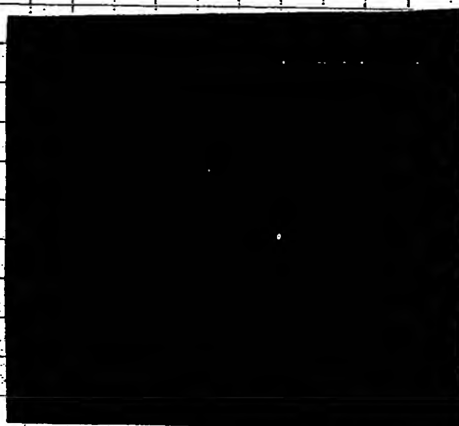
 HTPAN08 / HTPB44 in pD10

11/14/94

~~[illegible]~~

Run digest on gel - see pg 39.
with 1kb ladder

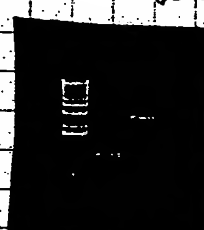
Run Digest on 0.8% LMP Gel
Run 80V 2 hrs



Cut out fragments
take picture

Use Qiaquick Gel Extraction Kit
- Add 900 ul QX1 Buffer
- Incubate 60°C 10 min
- Place in Spin Column
- Spin 60 sec - Remove flowthrough
- Add remaining Gel/QX1
- Spin 60 sec
- Remove flowthrough

Add 750 ul Buffer PE
Spin 60 sec
Remove flowthrough
Add 750 ul Buffer PE
Spin 60 sec
Remove flowthrough
Spin 60 sec
Transfer Column to fresh tube
Add 50 ul TE Heated 60°C
Spin 60 sec
- Run 4 ul on gel with 1kb ladder



looks good.

HTPAN08 / HTPB411 in PD10

11/14/94

Set-up ligations

	1	2	3	4	5
HTPAN08	4	—	—	—	—
HTPB411	—	4	—	—	—
PD10 1.1	—	—	4	—	—
PD10 2.4	1	1	1	1	—
10X Buffer	2	2	2	2	2
T4 Ligase	1	1	1	1	1
H_2O	12	12	12	16	17

Set up 2 sets - 1 for 16°C overnight
A- 1 set for RT 1 hr.

For A- 1 hr at RT

10 µl ligation
100 µl M15 Chem Competent Cells

Let sit on ice 1 hr
Heat 42°C 45 sec
Place on ice
Add 400 µl LB
Heat 37°C 1 hr
Plate onto LBT Amp + Kan plates
100 mm = 50 µl
@ 150 mm = 200 µl
Incubate 37°C O/N

11/15/94

Platings look OK
Very few colonies on Vector Alone
No colonies on Lig Rxn alone

42 HTPA8508 / HTPB411 in PD10

11/15/94

PCR Colonies from 1, 2 & 3 into LB + Amp + Kan.
Incubate 37°C 4 hrs.

Run PCR Rxns

HTPA8504		HTPB411 S#5	OLD
3' Xba New	0.2	3' Xba New	0.2
5' Bam New	2	5' Bam New	2.5
10x dNTP	3.2	10x dNTP	3.2
10x PCR	3.2	10x PCR	3.2
H2O	22	H2O	20.7
Taq	0.2	Taq	0.2
Culture	2	Culture	2
	32		32

⊕ Control
PCR'd insert -
⊖ Control LB + Amp + Kan
with vector alone Colonies
take both sets of primers + rxn mix
NO contamination by fragments

Run PCR Program #69.

95°C	5 min	
95°C	20 sec	30x
55°C	20 sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold.	

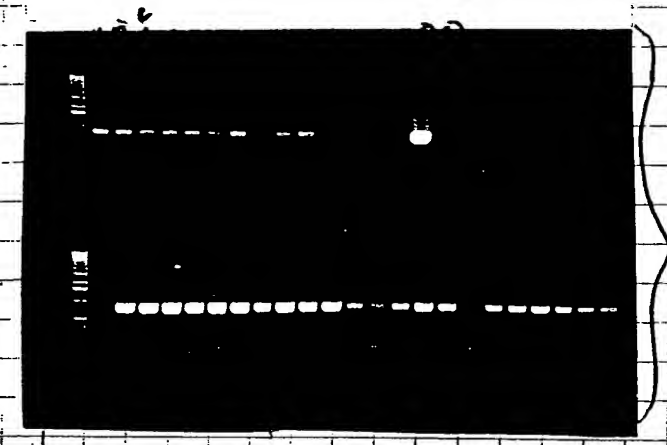
Run 10 ul on gel with 1 Kb ladder
use 1% Agarose gel in TBE

pg 47

HTPAN08/HTPB411 in PD10.

(pg 42)

11/15/94



HTPAN08S04

HTPAN08S04
-looks good.
HTPB411S05
Nothing to Re plate / Captains from 16°C O/N.

HTPB411S05 PD10

HTPAN08S04 -
Incubate 5ml TB+ Amp+Kan with 15ml of culture 2-11
Incubate 37°C O/N.

With ligation Rxns left at 16°C O/N

Thaw MS chemically competent cells on ice
To ~~50~~ 100µl of thawed cells add
10µl of ligation Rxn
~~to~~ (tube #6 add 10ng PD10 ~~[illegible]~~ plasmid DNA as ⊕ control)
Incubate on ice 1 hr.
Heat 42°C 45sec
Place on ice
Add 400µl LB
Incubate 37°C 1 hr.
Plate onto LB+Amp+Kan plates
~100mm plates ~ 50µl
~150mm plates ~~200~~ 250µl
Incubate 37°C O/N.

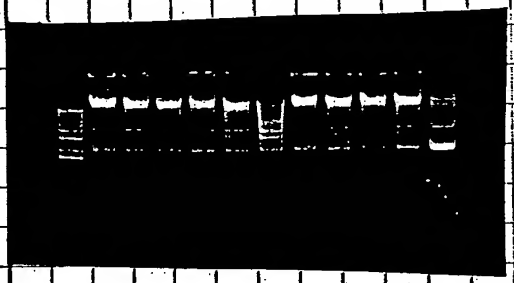
48 HTPB411 & HTPAN08 pD10

11/16/94

Boiling Mini preps

Spin cultures 2min
Remove Supernatant
Resuspend pellet in 750ul STET
+ RNase + Lepozyme
Boil 45sec
Spin 10 min
Remove Pellet
Add equal Volume (750ul) of
13% PEG / 1.6M NaCl
Mix Well
Spin 10min
Remove Supernatant
Add 1000ul 70% Ethanol to coarse
pellet
Spin 5min
Remove ~~pellet~~ supernatant
Allow pellet to dry slightly at RT.

Resuspend in 200ul TE
Run 2ul on gel with 1Kb ladder
and pD10



plasmid looks good.
Looks like alot of chromosomal.

Digest with BamHI to see if insert will "Pop" out
N800 bp

pg 53

HTPAU08 + HTPB4/1 1→ PDO.

pg 48

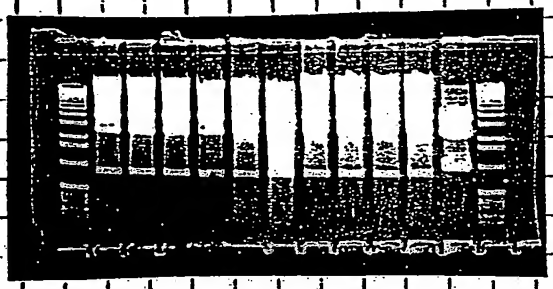
11/16/94

DNA	5ul
H2O	17.1
10x #2	2.5
Bam	0.2
Xba	0.2
	25ul

Incubate 37°C 4hrs
Run on gel with 1 Kb ladder
Digest pPIO - Should appear 1.1 Kb fragment

looks like allele
Contact
Submit for Sequencing
with PB10 5'
primer

FASPD01-10 RP01



Setup ligation for HTPB4/1

Fragment	16
Vector	1
T4 ligase	1
10X Buffer	2
	20ul

Incubate RT 5 hrs

Thaw MI5 cells on ice
To 100ul of MI5 chemically Competent cells Add 10 ul of ligation Reaction + Set up all of ligation.
Incubate on ice 1 hr
Heat 42°C 45sec
Place on ice
Add 900 ul LB
Incubate 37°C 1 hr
Plate 250 ul onto LB + Amp + Kan a plates - 150 mm

54 HTPAN08 + ~~HPRGG4~~ / HTPB411 in PDI0

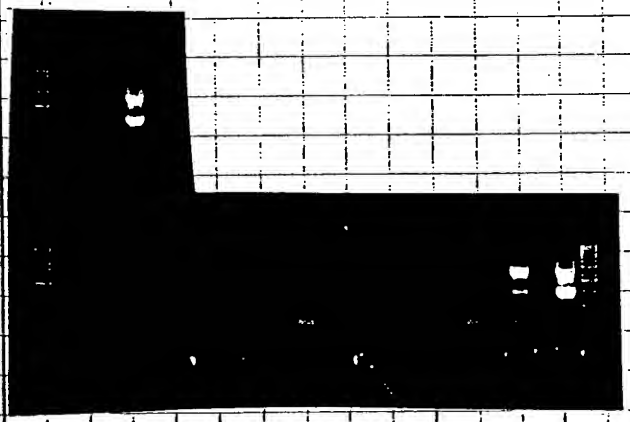
11/17/94

Some Colonies grew.
Pick into 200 µl LB + Amp + Kan
in 96 well dish.
Incubate 37°C 4hrs
PCR -

3'Xba	0.2
5'Bam	2.5
10xPCR	3.2
10xdNTP	3.2
Taq	0.2
H_2O	20.7
Culture	2
	32.

PCR Program #69
use HTPB411 5'5 3'Xba new / 5' ~~Bam~~ New
fragment as positive Control

Run 10 µl on gel with 1kb ladder.



looks like 1 positive !!!

Inoculate
50 ml TB + Amp + Kan
with #20
Incubate 37°C
O/N

11/18/94

Qiagen Midi prep -
Spin Culture 4.5K 15min
Pour off supernatant
Resuspend in Tris P1

11/18/94

Add 7ml P2
mix gently
Add 7ml P3
mix
Sit on ice 20min
Spin 4.5 K 15min
Equilibrate TIP-100 with 5ml QBT
Apply supernatant to tube through Kim wipe
Allow to flow through
2X Wash Column 10ml QC Buffer
Elute DNA 10ml QF
Add 7ml isopropanol (0.7 volumes)
Mix well
Spin 8K 30min
Remove supernatant
Wash 10ml 70% Ethanol
Spin 8K 15min.
Pour off supernatant carefully.
Let pellet dry at RT
Resuspend in 300ul TE.
Run gel on gel with 1Kb ladder

Dilute 1:200 in H_2O
Read OD 260/280

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm
1	0.0557	0.0350	0.0031	1.6927	0.5908

a Stonyfied

HTPB411
PD10

~ 110 ug total

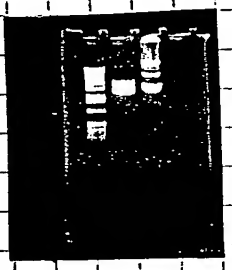
Digest with Bam / Xba to pop out 2.4 Kb 2.5 Kb fragment

DNA	4
H_2O	13.6
10X	2
Bam	0.2
Xba	0.2

Incubate 37°C
2 hrs

56 HTPAD08 + HTPB411 in PD10

11/18/94 Run 5ul on gel with 1/6 loading dye & 1ul of Undigested.



11/28/94

Made New PAZ Primers 5'end for HTPAD08.

279A 5' Bam 51bp New 7.1pmol/ul
279B 5' Bam 185bp New 8pmol/ul
Use 3' Asp from pervious PAZ cloning 7690

PCR.

5' Bam	3
3' Asp	0.5
10x PCR	10
10x dNTP	10
H_2O	75.2
Taq	0.3
DNA (10ng/ul)	1
	100

[6X]

PCR Program #69.

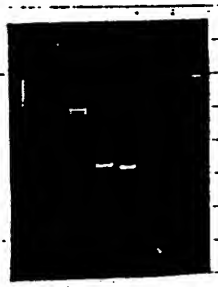
95°C 5 min
95°C 20sec
55°C 20sec
72°C 1min
72°C 7 1/2 min
11°C Hold

25X

HTPAN08 + HTPBY11

11/21/94

Rem 5ul on gel with 1Kb ladder



Add equal volume PEG/NaCl-mix
Spin 10 min
Remove supernatant
Wash pellet 1ml 70%
Ethanol
Resuspend in 30ul TE
Rem 2ul on gel with 1Kb ladder

Digest fragment
Bam/Xba

DNA	20
H_2O	23
10X#2	5
Bam	1
Asp x ba	1
	50ul

Digest O/N
Store -20°C

11/28/94

Digested w/ x ba by mistake.
Red Digest with

Bam/Xba Asp.

DNA	20
H_2O	23
10X#2	5
Bam	1
Asp	1
	50ul

Incubate O/N 37°C.

58 HTRA1 08 + PA2 lig

11/30/94

Isolate on 0.8% LMP Gel & Qiaex Clean.
see pg 62

Set up ligations

185 bp Bam/Asp	10	—	—
51 bp Bam/Asp	—	10	—
PA2 Bam/Asp	1	1	1
10x Buffer	2	2	2
T4 Ligase	1	1	1
H2O	16	16	16
	20	20	20

Incubate 16°C O/N.

12/1/94

Transform into M15 cells

Thaw M15 Chem. Competent Cells
To 60 ul of ligations add 100 ul of cells
Incubate on ice 1 hr
Heat 42°C 45 sec
Place on ice
add 400 ul LB
Incubate 37°C 1 hr.
plate 100 ul on 100 mm plates
300 ul on 150 mm Plates

12/2/94

M15 cells Contaminated? pg 67

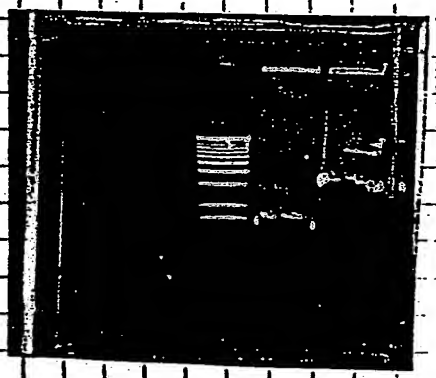
62 | PD10 B/HIII / HTPAN08 Frag / DNaseI frag

11/30/94

Gel isolate fragment on
0.8% 1 MP gel

Also Run Bam
1 HTPAN08 185 bp Bam/Asp
2 HTPAN08 511 bp Bam/Asp
3 DNaseI Bam/Xba
4 PD10 Bam/HIII
Wash 1 Kb ladder

Run 80V 1½ hrs
Cut out bands & Take Picture



Gene Clean fragments
Add 1ml NaI
Heat 55°C 5 min
Add 5 ul Glass milk
Incubate RT 2 min
Spin 10 sec
Remove Supernatant
3x { Resuspend pellet in
500 ul Wash Buffer
Spin 10 sec
Remove Supernatant }

Spin 10 sec
Remove all supernatant possible
Resuspend pellet in 20 ul TE for
HTPAN08 511 bp + 185 bp DNaseI
Resuspend pellet in 40 ul TE PD10 Bam/HIII
Incubate 55°C 2 min
Spin 10 sec
Transfer Supernatant to fresh tube.
Re elute.
Run 2 ul on 1% gel with 1 Kb ladder
Store at -20°C

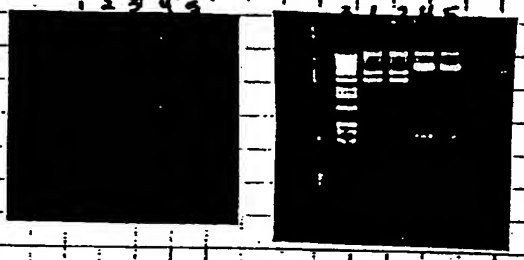
HTPAN08 — see pg 58
DNaseI — see pg

HPRCC91, HE2014Z2

pg60

12/5/94

1. HE2014Z2S15
2. S14
3. 1kb ladder
4. HPRCC91S14
5. S15



Decided to use a 3' fragment for screening enriched.
Inoculate 150 ml TB + Amp.
HE2014Z2S14
HPRCC91S14

12/6/94

Qiagen Maxi prep of: HE2014Z2S14
HPRCC91S14

Spin cultures 4.5K 15min
Pour off supernatant
Resuspend pellet in 10ml P1+RNase
Let sit at RT 5min
Add 10 ml P2 mix gently
Let sit at RT 5min
Add 10ml Cold P3 & incubate on ice 20min
Spin 4.5K 15min
Equilibrate Column 10ml QBT
Apply supernatants to Columns
Wash 2x 30ml QC
Elute 15ml QF
PPT with 10.5ml Isopropanol
Spin 9K 30min
Pour off Supernatant
Wash 10ml 70% Ethanol
Spin 9K 10min
Pour off supernatant

Heidi Pch
12/5/94

HTPA008504 516bp/185bp in PA2

pg 58

12/2/94

The 4g Rxn alone & MI5 cells alone produced colonies — Don't use MI5 — use DH5α.

Thaw Chemically Competent DH5α cells on ice

To 100 ul Cells add 10 ul of ligation

Incubation ice @ 1 hr

Heat 42°C 45 sec

Put on ice

Add 400 ul LB

Incubate 37°C 1 hr

Plate 200 ul onto 150 mm LB+Amp

Incubate 37°C O/N.

12/5/94

Take plates out of 37°C

Put at 4°C

12/6/94

Pick into 200 ul LB+Amp.

Incubate 37°C a/N w/ shaking

12/7/94

PCR To test for inserts

516bp		40X	185bp		40X
5' Bam 51	0.2	8	5' Bam 185	0.2	4
3' Asp	0.2	8	3' Asp	0.2	4
10x dNTP	3.2	128	10x dNTP	3.2	64
10x PCR	3.2	128	10x PCR	3.2	64
Taq	0.2	8	Taq	0.2	4
H2O	21	840	H2O	21	420
Cult	2		Cult	2	

68 HTPAN08S04 51bp/185bp in PAZ

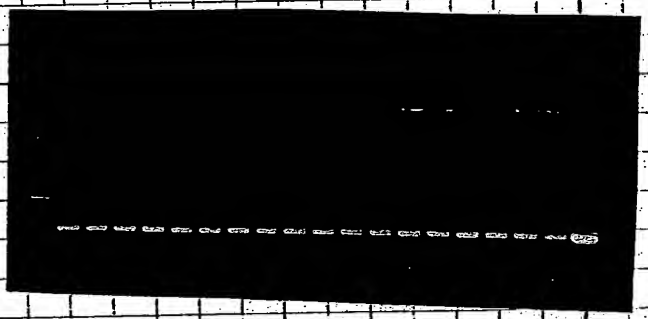
12/7/92

PCR proj # 66.

95°C	5 min	
95°C	20sec	30x
55°C	20sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

Run 10ul on 1% gel with 1Kb Ladder.

HTPAN08S04 51bp HTPAN08S04 185bp



Pick ⊙ HTPAN08S04 185 bp into ~5 ml TB+Amp Incubate 37°C O/N w/aeration

12/8/94

Boiling mini preps

Spin 2ml Culture 5 min

Remove Supernatant

Resuspend pellet 750 ul STET + RNase + Lysozyme.

Pg 84

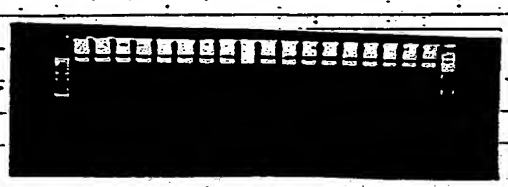
HTPH1108 PA2

pg 68

12/8/94

Boil 1 min
Spin 10 min
Remove pellet
Add 750 ul PEG/NaCl
Mix well
Spin 10 min
Remove supernatant
Add 1000 ul 70% Ethanol
Wash pellet
Spin 5 min
Remove supernatant
Allow pellet to dry at RT 10 min.

Resuspend pellet 20 ul TE
Run 2 ul on gel with 1Kb ladder + λ HIII



Digest with Xba

Try digesting with Bam/Asp.

		19K
DNA	4	
H_2O	22.6	429.4
10x T	3	57
Bam	0.2	3.6
Asp	0.2	3.6

Xba

Incubate 37°C O/N

Mia Pelle
12/2/94

12/9/94

Does not look like anything digested

Submit for Sequencing
RP06
RP19.

1/11/95

Remake inserts w/ new primers
for Both PA2 + PD10

PA2 has Bam HI site with
Kozac Seq.

51bp + 185 bp fragments

PCR	③ PD10 HTPAN08 51bp		④ PD10 HTPAN08 185bp		① PA2 HTPAN08 51bp		② PA2 HTPAN08 185bp
9113	20	9114	15	9111	18	9112	15
2742	2	2742	2	2742	2	2742	2
10X PCR	50		50		50		50
10X dNTP	50		50		50		50
Taq	2		2		2		2
H_2O	375		382		377		382
DNA	1		1		1		1

DNA - HTPAN08S04 plasmid DNA - 10ug/ul.

PCR using Program # 66

⊝ control
Hamby

pg 125

PQE60 Nco / Bam Vector

12/19/94

Digest PQE60 = 0.54ug/ul

DNA	4 ul
10x #4	5
H2O	40
Bam	0.5
Nco	0.5
	50

Incubate 37°C O/N

12/20/94

Run 2 ul on gel with 1Kb ladder

Looks good.
Add 5 ul 10x SB
Run on 0.8% LMP gel.
80V 1½hr
Cut out fragment.
Take Picture

Gene Clean.
Add 900 ul NaI
Heat 55°C 5 min
Mix well.
Add 7 ul Glass milk
Mix well
Incubate at RT 5 min
with occ. mixing
Spin 10 sec
Remove Supernatant
Resuspend pellet 500 ul
3x Wash Buffer
Spin 10 sec

HTPAN08/HTPB411 in PQE60

12/20/

PCR
HTPAN08S04 5' NcoI / 3' BglII
HTPB411S15 5' BspHI / BamHI
for ligation onto PQE60

	HTPAN		HTPB4
5' NcoI	20	5' BSPHI (51bp)	20
3' BglII	4	3' Bam	4
10x dNTP	30	10x dNTP	30
10x PCR	30	10x PCR	30
H_2O	234	H_2O	238
Taq	1	Taq	1
DNA (1ng/ul)	1	DNA (1ng/ul)	1
	300		300

Set up Rxn w/o DNA (30ul vol total)

PCR: 10x 30ul Rxns.

Prog 66. Run 5ul on gel with 1kb ladder.

95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1min	
72°C	7 1/2 min	
4°C	hold	

5
5
39ul
0.5
0.5
50ul

Add equal Vol PEG/NaCl to ppt. - mix well
Spin 10min
Remove Supernatant
Wash 1X 70% Ethanol (100ul)
Spin 5min
Remove supernatant.
Allow pellet to dry at RT 10min
Resuspend in 10ul TE

100 HTPA908 | HTPB4H in PQE60

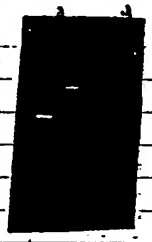
12/20/94

Run 50µl on 0.8% LMP gel
80 V 1½ hrs

Cut out fragments

Gene clean
Resuspend - Total 40µl TE

Run 2µl on gel with 1Kb ladder



looks good -

Set up digestions:

DNA	30µl
H_2O	13
10X #4	5
Enzymes	1/1
	30µl

For HTPA908 - Nco I / Bgl II
HTPB4H1 Bgl II / Bam -

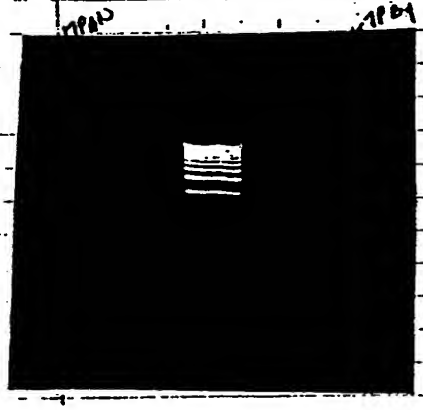
Incubate 37° C O/N.

12/22/94

Add 5µl 10X SB -
Run on 0.8% LMP gel with
1Kb ladder
80 V 1½ hrs

HTPBN08 | HTPB411 in PQE60 | 101

12/22/9



Gene Clean

Resuspend and Elute in 40 ul Total

Run 2 ul on gel with 1Kb ladder

Set up ligation

	①	②	③	④	⑤	⑥
HEPAN08S04 5'Nco/5'BglII	4	—	4	—		
HTPB411S15 5'Nco/3'Bam	—	4	—	4	—	
10x	2	2	2	2	2	2
H_2O	12	12	13	13	16	1
T4	1	1	1	1	1	1
PQE 60 Nco/Bam	—	1	—	—	—	
PQE 60 Nco/BglII	1	—	—	—		

Incubate 4°C

1/3/95

Transform M15 sup b cells
Chemically competent (Hong)

Thaw cells on ice
To 100 ul of cells add Mix & ligation
To 1 tube add 10 ul PQE60 Dig Prep
Tube add 10 ul of H_2O only

102 HTRANS8 [HTPE4II in PQE60

1/3/95

Incubate on ice 1 hr
Heat 42°C 1 min
Let sit on ice 2 min
Add 400 µl LB
Incubate 37°C 1 hr.
Plate onto 2 B+ Amp + Kan plates.

1A – 200 µl, 300 µl
1B – 100 µl, 400 µl
2A – 200 µl, 300 µl
2B – 100 µl, 400 µl
3 – 300 µl
4 – 300 µl
5 – 300 µl
6 – 300 µl ligation Rxn.
7 – PQE60 10 ng 100 µl
8 – M15 cells alone – 300 µl

Spread Evenly with Beads
Incubate 37° O/N.

1/4/95

All plates grew –
Even M15 cells alone and ligation Rxn alone
Cells are Contaminated!
Told Lang – Said he would test Cells & make New.

Try Redoing ligation with
HTRANS8504 185 bp fragment.

(Pg 107)

HTPAN08S04 185 bp Fragment 103

12/23/94

PCR HTPAN08S04 185 bp fragment
for PQE60 Nco/Bgl II

DNA	1 ul
H2O	237
10x PCR	30
10 mM dNTP	30
Taq	1
5' NcoI 185bp	20
3' Bgl II	4
	300 ul

Use H2O only as ⊖ Prep.

Split 10 tubes

PCR Program #66.

95°C	5 min	
95°C	20 sec	30x
55°C	20 sec	30x
72°C	1 min	30x
72°C	7 min	
4°C	hold	

Store 4°C

Run
PCR (10 ul with) 1 Kb ladder 1/3/94

[redacted]

Add equal Volume 13% PEG/
1.6 M NaCl
incubate on ice 10 min
mixing well
Spin @ 10 min
Pour off supernatant.

104 | HTPAN08S04 | 185bp Fragment

1/3/95

Wash pellet in 700 µl 70% Ethanol.
Spin 5 min.
Pour off supernatant
Allow pellet to dry at RT
Resuspend pellet in 200 µl TE

Now Set up Digestion

DNA	35
H_2O	8
10X #4	5
Pst	1
Bgl II	1
	50 µl

Incubate 37°C O/N.

1/4/95

Run LMP Gel - 0.8% in TAE.
80V 1½ hrs

[redacted]

Cut out fragment from gel and place in Tube
Take picture of gel.

2 Tubes

1 Tube use 6M NaI from Bio 101
Other Tube use 6M NaI made new

Add 1000 µl NaI
Heat 55°C 5 min

(pg 107)

~~Vector Soup~~ 186 bp Fragment
HTLAD31 PER Protein

1/2/95

PER-Protein
Digest HTLAD31 = 0.25 ug/ul

① Xho I / EcoRI
② Sac I / Kpn

①		②	
DNA	6	DNA	6.
H2O	20.6	H2O	20.6
10X #2	3	10X #4	3
Xho	0.2	Kpn	0.2
EcoRI	0.2	Sac	0.2
	30ul.		30ul

Incubate 37°C O/N.
Submitt for sequencing -

1/4/95

Run 10ul on 1% TAE gel
with 1Kb ladder

1- Xho I / EcoRI ~4.7Kb.

2- Kpn / Sac ~800 bp to ~600 bp

Insert looks ~ 11.6 Kb

Make Primers -
RPD6 & FPD5

1/9/95 Recieved RP06 & RP04.
Submit for sequencing with other RP02, RP03 and Reverse & Forward

1/11/95 Recieved FP05.
Submit for Seq RP06 & FP05

1/13/95 Large 3' untranslated region
~1.5 Kb long.
About 1.2 Kb is untranslated.

HTPA1084 SAU 1856p Fragment

(pg 104) & pg 102

1/4/95

Mix well to make sure all of gel is dissolved.
Add 8µl Glass milk & mix.
Incubate at RT 2 min w/ occasional mixing
Spin 10 sec
Pour off supernatant
3x { Resuspend pellet in 400µl Wash Buffer
Spin 10 sec
Pour off supernatant }
Spin 10 sec
Remove as much of Supernatant as possible
Resuspend pellet 30µl TE
Heat 55°C 2 min
Spin 10 sec
Transfer Supernatant to fresh Tube.
Resuspend pellet in 20µl TE
Heat 55°C 2 min
Spin 10 sec
Transfer to tube
Run 2µl on gel with 1 kb ladder

1- using Gene Clean Kit for Nal

2- using Home made GH Nal

Both look good..
Ready for Ligations

1/5/95

Set up ligation for
HTPA084 HTPB411 in PUC 19

108 HTPAN08 + HTPBY11 in PQE60

1/5/95

	①	②	③	④	⑤	⑥	⑦	⑧	⑨
HTPAN08504 5'Nco/3'BglII	6			6					
HTPAN08504 (Bbp 5'Nco/3'BglII		6			6				
HTPBY11S5 5'BspHI/3'BamHI			6			6			
10x Buffer	2	2	2	2	2	2	2	2	2
PQE60 Nco/BglII	2	2					2		
PQE60 Nco/BamHI			2					2	
H_2O	9	9	9	11	11	11	15	15	17
T4 DNA Ligase	1	1	1	1	1	1	1	1	

Make Cocktail

		9x
10X Buffer	2	18
H_2O	9	81
T4 DNA lig	1	9
	12	

Store ligations at 4°C till first M15 cells are found

1/9/95

STEVE said to transform into XL-1 Blue
If plasmid is correct - it can transform into M15 cells.

Thaw XL-1 Blue Chemically competent cells on ice
To 10ul of ligation add 100ul thawed cells
Incubate on ice 1 hr - as ⊖ control use 100ul cells only
Heat Shock 42°C 45sec

P115

Maxi Prep HTPAN08504/HMEAA88

1/4/95

Inoculate 200 ml TB+Amp
from frozen stocks of
HTPAN08504
HMEAA88A.

Incubate 37°C w/aeration
overnight.

1/5/95

Qiagen Maxi Prep

Spin Cultures 4.5K 15min
Pour off Supernatant
Resuspend pellet in 10ml P1 +
RNase
Incubate RT 5min
Add 10 ml P2
Incubate RT 10min
Add 10 ml P3 - ice cold
Incubate on ice 20 min
Spin 4.5K 15min
Equilibrate Tip-500 with 10ml
QBT
Apply Supernatant to tip through
Kim wipe
allow to flow through
Wash Column 3x
30ml QC
Elute DNA - 15 ml QF
Add 10.5 ml (add 0.7 times the volumes)
Mix well
Spin 8K 30min
Pour off Supernatant
Wash pellet 15ml Cold 70% Ethanol

112 Maxi Prep HRAN08504, HMEAA88

1/5/95

Spin 8K 15min
Pour off supernatant
Allow O/N pellet to dry
Dry o/n at Room Temp -
w/d. parafilm w/holes covering

1/6/95

Resuspend pellet in 400 ul TE
Run 1ul on gel with 1Kb ladder
& λ Hind III Marker

Dilute 1:100 in H_2O
Read $OD_{260/280}$.

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 320.0 nm	280.0 nm / 260.0 nm	
1 [illegible]	0.0402	0.0319	0.0231	1.9374	0.5161	0.2 ug/ml
2 [illegible]	0.2051	0.1314	0.0315	1.7380	0.5754	1.03 ug/ml

looks like HMEAA88 is all chromosomal

1/9/95

Steve said to try transforming with Boiling prep DNA to get a clone

(pg 108)

1/9/95

Set on ice 2 min
Add 400 µl LB.
Incubate 37°C 1½ hrs.
Plate 200 µl onto LB + Amp
150mm plates

Transform ligation from 12/22
400.

Incubate 37°C O/N
Plate M15 Cells on LB + Amp
LB + Kan
LB + Amp / Kan.

1/10/95

Plates look good.
No Contamination

M15 Cells
Grew on LB + Kan
& on LB + Amp + Kan
Not on LB + Amp.
∴ LB + Amp / Kan plates Do not have Amp or enough amp so contamination can ~~be~~ seen.

Can use M15 cells - Not Contaminated

Pick - ~~[illegible]~~ colonies onto LB + Amp in 96 well dish.

HTPBY11			HTPAN.08.		
12/22	2A -	36	12/22	2	48
12/22	2B -	44	1/5	1	48
1/5	3 -	25	1/5	2	48

pick pQE60 Vector + PAZ vector as controls

116 HTPAN08/HTPBU11 in POE6O

1/10/95

Incubate 37°C w/aeration 5hrs
PCR using Universal primer
as well as the 2 external Primers

HTPBU11 + POE6O	
FP20	0.5
2887	0.4
2886	0.2
10xdNTP	3.2
10x PCR	3.2
H_2O	18.7
Taq	0.2
Culture	2
	32

HTPAN08 5 lbs + POE6O	
FP14	0.2
2885	0.2
2865	0.2
10xdNTP	3.2
10x PCR	3.2
H_2O	17
Taq	0.2
Culture	2
	32

HTPAN08 185 + PO
FP4 0.2
2867 0.2
2865 0.2
10xdNTP 3.2
10x PCR 3.2
H_2O 28.8
Taq 0.2
Cult 8.0
32

PCR - ⊕ control Plasmid DNA
⊖ control LB + Amp alone

PCR Prog 66

95°C	5min	
95°C	20sec	30x
55°C	20sec	
72°C	1min	
72°C	7 1/2min	
4°C	Hold	

Set up Rxns
Let Cultures
grow 37°C O/N
@ aeration

1/11/95

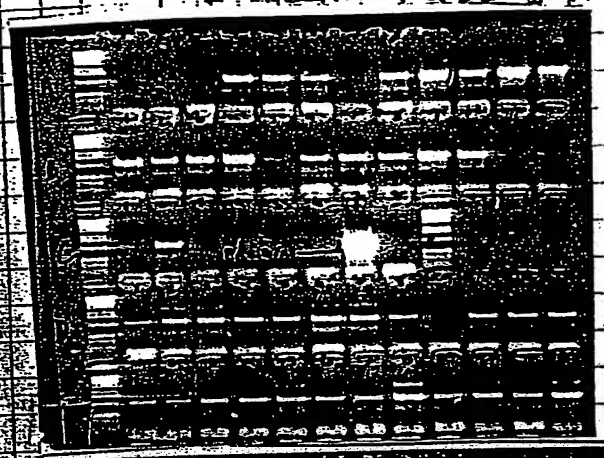
Run 10µl of PCR Rxn on a 1.3% Agarose gel in TAE with 1Kb ladder

HTPB411 in PAZ / HTPAN08 + HTPB411 in PQE60

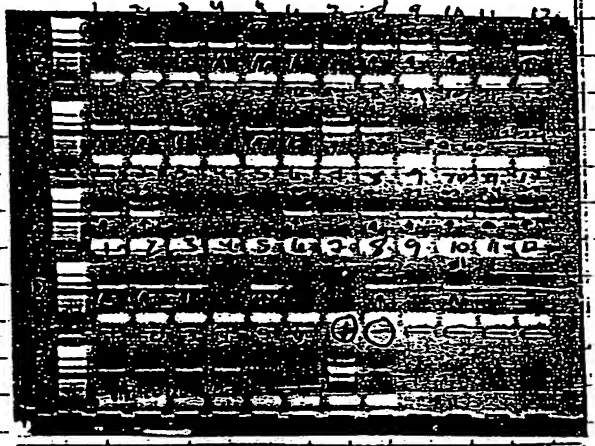
pg 110 pg 116

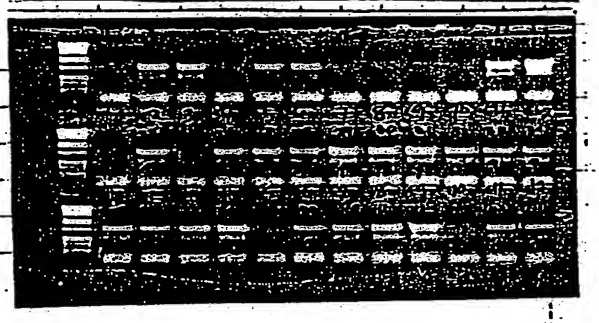
1/11/95

① HTPB411 + PAZ B-E
HTPB411 + PQE60 D-H

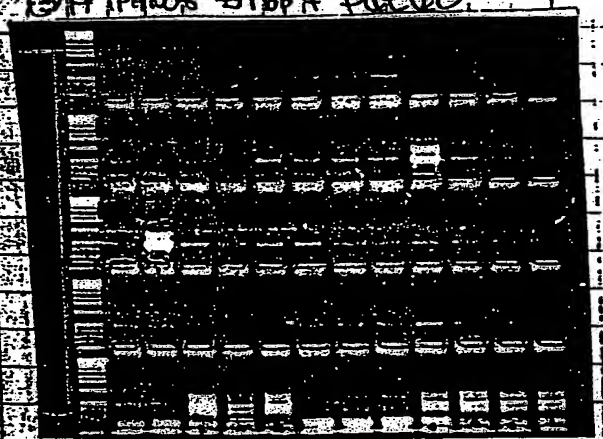


② HTPB411 + PQE60 B-E
HTPB411 + PAZ F-H

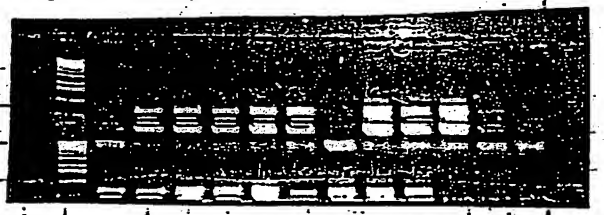


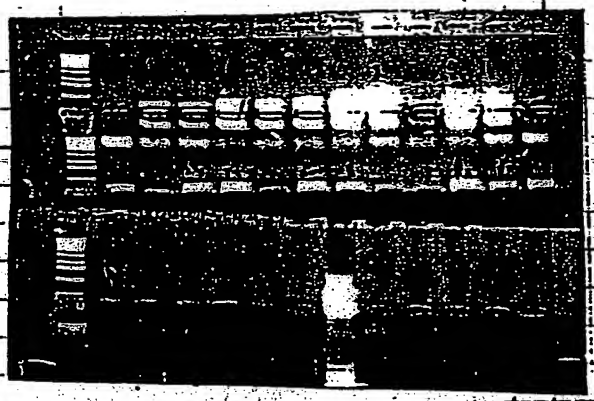


③ HTPAN08 Stop + PQE60



④ HTPAN08 18Stop + PQE60





120 | HTPB411 PAZ | HTPB411 & HTRAN in PQE60

1/10/95

Inoculate EB TB + Amp 5ml ea
do Boiling preps.

① HTPB411 + PAZ
1, 5, 7, 8, 12, 16, 17, 18, 22, 23, 24, 29, 30, 31, 32, 41, 42, 43

② HTPB411 + PQE60.
2, 6, 7, 19, 23, 24, 29, 38, 41, 45, 48, 57, 58, 66, 69, 73, 74, 79

③ HTRAD085bp + PQE60.
1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 14, 21, 23, 26, 27, 31, 33, 34

④ HTRAD085856bp + PQE60
5, 6, 7, 8, 11, 13, 15, 16, 17, 20, 21, 22, 23, 24, 25, 26, 28, 32

Incubate 37°C w/ Aeration overnight

1/12/95

Boiling mini Preps

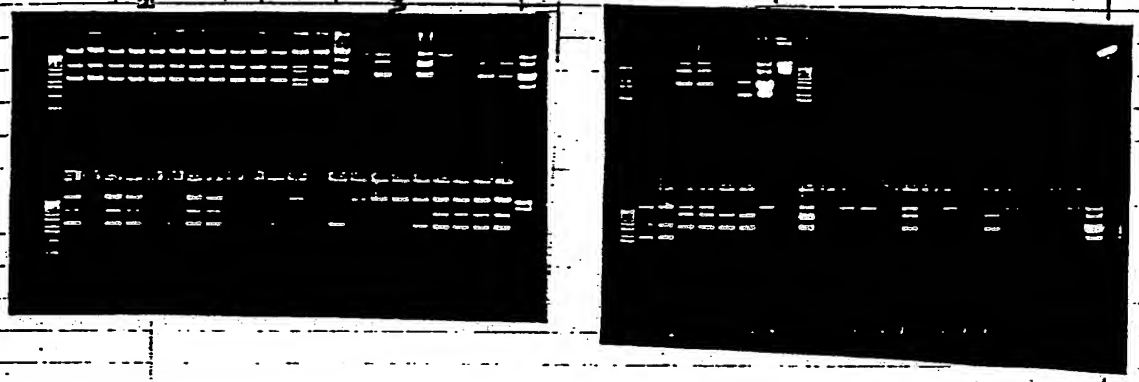
Spin 2ml of culture 2min
Remove Supernatant
Resuspend pellet in 750 µl STET +
RNase & Lysozyme
Boil 1min
Spin 10 min
Remove Pellet
Add Equal Volume ~ 750 µl of 13% PEG 8000
1.6 M NaCl
Mix Well
Spin 10 min
Remove Supernatant
Wash Pellet 1000 µl 70% Ethanol
Mix

HTPB411 PA2 / HTPB411+HTPAU08 : PQEGO.

4/12/95

Spin 5 min
Remove Ethanol
Allow pellets to Dry at RT for 30 min
Resuspend pellets in 100 ul TE

Run 2 ul of Mini preps on 1% TAE gel with 1Kb ladder + PA2 & PQEGO



mix up in labeling - #1 is really #3
#2 is really #4
#3 is really #1
#4 is really #2

Set up digestions

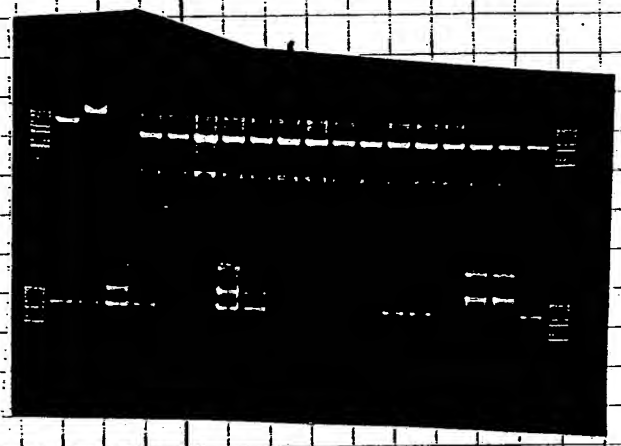
For PQEGO.			For PA2		
EcoRI / HIII		55X	Bam / Xba		20X
DNA	4		DNA	4	
10X	2	110	10X	2	40
H_2O	13.6	748	H_2O	13.6	272
EcoRI	0.2	11	Bam	0.2	4
HIII	0.2	11	Xba	0.2	4

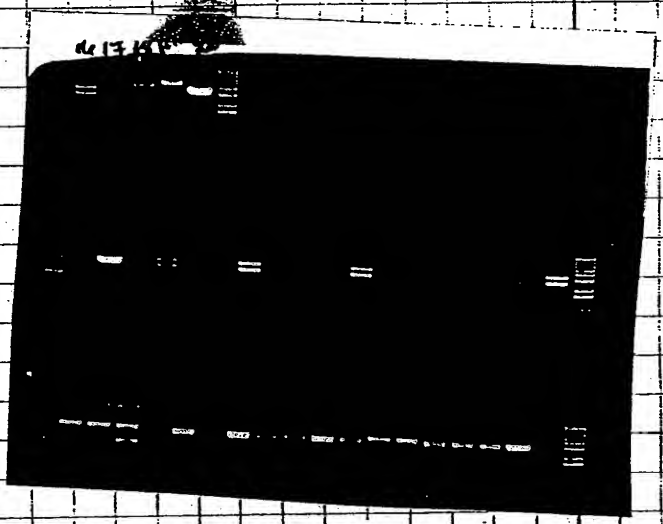
Incubate 37°C O/N
Digest HTPAU08504 HTPB411515
PA2 & PQEGO

122 HTPB4H PAR / HTPAN08 + HTPB411 PCR'd

1/13/95 1,2,4 - Eco / HIII 3, Bam / Xba digested

Run 10ul on gel with 1kb ladder





Looks like
4 - HTPAN08 185bp + PCR60
1 - 17 correct - Sequence 4.
2. HTPB411 in PCR60
1-4, 6, 8 - 16 looks good Seq 4.

143 Need to be digested w/ correct Enzymes

Submit 4 & 2 (4 of each)
#4 with RPO16 & FP16
#2 FP23
for Sequencing

1/18/95

Clean up DNA of 4-1, 4-2, 4-3, 4-4
2-1, 2-2, 2-3, 2-4
2x Phenol
2x CH Cl3 : IAA
Ethanol PPT - Wash
Resuspend in 80 ul TE - use to transform into M15 cells

pg 127

HTPAN08 PAZ/PDIO

pg10

95°C 5min
95°C 20sec
55°C 20sec } 30x
72°C 1min
72°C 7.5min
94°C Hold

Run 5µl on gel with 1kb ladder 11/12/95

3 - HTPAN08 351 bp for PAZ
4 - HTPAN08 185 bp for PAZ
1 - HTPAN08 351 bp for PDIO
2 - HTPAN08 185 bp for PDIO

4/5ths

1

Precipitate, Add equal Volume
3% PEG/NaCl
Spin 10 min
Wash pellet 1000µl @ 70% Ethanol
Spin 5 min
Dispose Supernatant
Set up digestions

DNA (PCR Fragment)	20
10 x #2 Buffer	4
H_2O	15
Bsm	0.5
Xba	0.5
	40µl

Incubate 37°C Overnight

 HTPAN08 PA2 | PD10

1/13/95

Run All of digestion of 0.8% Agarose LMP Gel

Cut out gel fragments.
Take picture

1 - HTPAN08 51bp } PD10
2 - HTPAN08 185bp }
3 - HTPAN08 51bp } PA2.
4 HTPAN08 185bp }

Gene Clean.
Add 800 µl NaI
Heat 55°C 5 min.
Add 8 µl Glass Milk
Mix well
Incubate at RT 5 min
w/ occasional mixing
Spin 10 sec
Remove Supernatant

3X [
Add 500 µl Wash Buffer
Resuspend pellet
Spin 10 sec
Remove Supernatant
]

Spin 10 sec
Remove Supernatant
~~Supernatant~~
Resuspend pellet in 20 µl TE

2X [
Heat 55°C 1 min
Spin 10 sec
Transfer Supernatant to fresh tube
]

Run on gel with 1 Kb ladder and 1 µl of PA2 B/X 1 µl PD10 B/X

1 HTPAN08 51bp PA2
2 HTPAN08 185bp PA2
3 PA2 Bam/Xba
4 HTPAN08 51bp PD10
5 HTPAN08 185bp PD10
6 PD10 Bam/Xba

HTPAN08/HTPB41 PAZ/PD10

pg 122

1/13/95

Set-up ligations

	1	2	3	4	5	6	7	8	9	10	11	12	13
HTPAN08 SI PAZ	6	—	—	—	—	6	—	—	—	—	—	—	—
HTPAN08 SS PAZ	—	6	—	—	—	—	6	—	—	—	—	—	—
HTPAN08 SI PD10	—	—	6	—	—	—	—	6	—	—	—	—	—
HTPAN08 SS PD10	—	—	—	6	—	—	—	—	6	—	—	—	—
HTPB41 PD10	—	—	—	—	6	—	—	—	—	6	—	—	—
10X Buffer	2	2	2	2	2	2	2	2	2	2	2	2	2
H_2O	9	9	9	9	9	11	11	11	11	11	15	15	17
T4 Ligase 1U/µl	1	1	1	1	1	1	1	1	1	1	1	1	1
PAZ Bam/Xba	2	2	—	—	—	—	—	—	—	—	2	—	—
PD10 Bam/Xba	—	—	2	2	2	—	—	—	—	—	—	2	—

Set up ligations on ice
Incubate 16°C over the weekend

over

1/14/95

Thaw MI5 chemically competent cells on ice
To 100µl of Cells add 10µl of ligation (from 1/13/95) and 1µl of DNA (from pg 122)
Do 1 tube of MI5 cells only / 1 of long PD10
Incubate on ice 1 hr
Heat 42°C 45sec
Place on ice
Add 400µl LB
Incubate 37°C 1½ hrs
Plate onto LB + Amp 150mm plates
- ligations ÷ 1-5 plate 100+300µl
- Plasmid DNA - plate 50µl
- Ligation control 6-13 plate 300µl
- MI5 cells plate 300µl
- MI5 cells + PD10 plate 100µl

Incubate 37°C O/N

HTPBY11 + PHTPAN08

1/16/95

HTPBY11+PA2
1/10/95 Transformation
Pick 96 colonies into LB+Amp

HTPAN08 51bp + PQE60
1/10/95 Transformation
48 from 1/5/95 ligation
48 from 12/22/94 ligation
into 200ul LB+Amp
Incubate 37°C w/ aeration O/N.

1/17/95

Transformations worked well -
No colonies seen on fragment only plates
2-~~25~~5 colonies seen on Vector alone plate.
No colonies on - M15 cells alone
- Lig. Rxn alone.

Pick colonies into 200ul LB+Amp+Kan

Plate [1] (1) HTPAN08 51bp +PA2 - 48 A1→D12
(2) HTPAN08 185bp +PA2 48 E1→H12

[2] (3) HTPAN08 51bp +PD10 - 48 A1→D12
(4) HTPAN08 ~~185~~185bp +PD10 - 48 E1→H12

[3] (5) HTPBY11 + ~~PQE~~ PD10 - 16 A1→B4
~~PQE60~~ PD10 14
PA2 ~~18~~ 4

[4] 2-1 (12) } A1-A12
2-2 (3) } HTPBY11 + PQE60 B1-B3
2-3 (12) } in M15 cells C1-C12
2-4 (12) } D1-D12

HTPB411 + H TPAN08

1/17/95

E1-E12 4-1 (12)
F1-F12 4-2 (12)
G1-G12 4-3 (12)
H1-H12 4-4 (12)
} HTPAN08 185bp + PQE60 M15 cells

Incubate at 37°C w/aeration 4 hrs.

PCR - HTPB411 + PA2 & HTPAN08 51bp + PQE60

HTPAN08 51bp + PQE60		100X
2888	2	200
7409 FP16.	0.15	15
10x dNTP	3.2	320
10x PCR	3.2	320
H_2O	21.25	2125
Taq	0.2	20
Cult	2	
	32ul	30ul/Tube

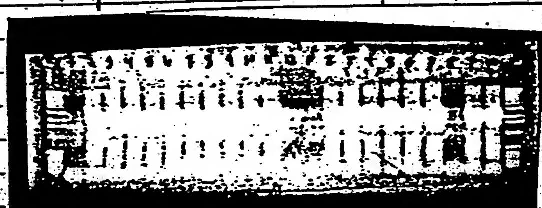
HTPB411 + PA2		100X
2741	0.2	20
7796 RPQ60	1.2	120
10x dNTP	3.2	320
10x PCR	3.2	320
H_2O	22	2200
Taq	0.2	20
Cult	2	
	32ul	30ul/Tube

PCR Prog. Col.
95°C 5min
95°C 20sec
55°C 20sec } 30X
72°C 1min
72°C 7 1/2 min
4°C Hold

Run 1% agarose gel with 1 Kb ladder

© control use 1 & Kanamycin only

1→D12
1→H12
2 D12
2 H12
2 B4
A12
B3
D12
D12



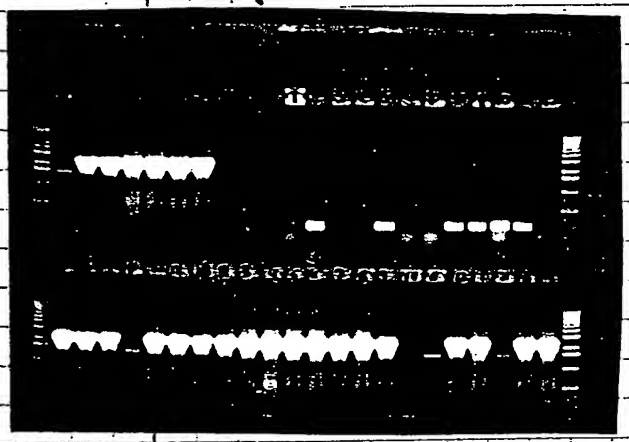
HTPB411 + PA2
2741 (3'Xba) + 7796 (RPQ60)

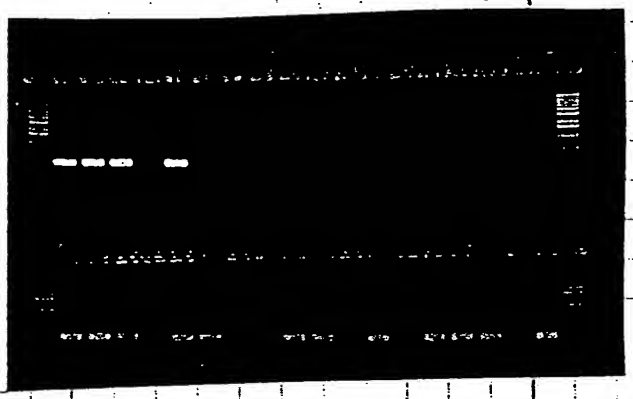
130

HTPB411 & HTPAN08

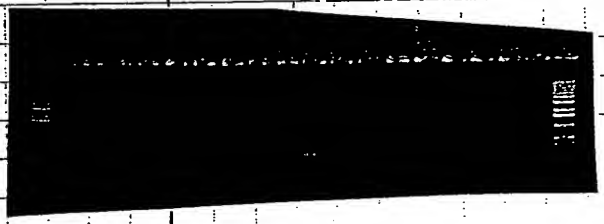
1/17/95

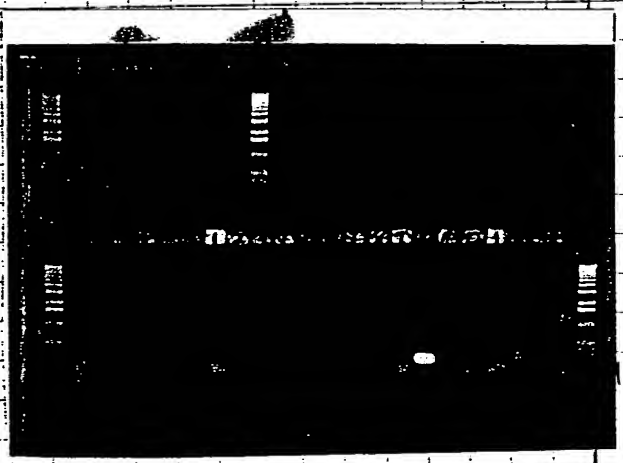
[HTPAN08 SIbp + PQE6O (2888 5'BspHI + 7409 FP11e)





HTPB411 + PA2
2741 (3'Xba1) + 7796 (RP06)





HTPAN08 SIbp + PQE6O
2888 (5'BspHI) +
7409 (FP11e)

Inoculate 5ml TB+Amp w.th.
Clones 1-9 from HTPB411+PA2
HTPAN08 SIbp + PQE6O

Incubate 37°C w/ aeration O/N

PCR Plates [1], [2], [3], [4]

HTPBHII & HTPAN08

1/17/95

Plate 1

① HTPAN08 51bp + PA2

		55X
9111	1.7	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
Taq	0.2	11
Cult.	2	
	32 µl	32µl/Tube

+control HTPBHII

② HTPAN08 185bp + PA2

		55X
9112	1.7	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
Taq	0.2	11
Cult.	2	
	32µl	30µl/Tube

③ Control PA2 Vector & LB Amp/Kan ④ Control HTPAN

Plate 2

③ HTPAN08 51bp + PD10

		55X
9113	1.4	77
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	21.9	1204.5
Taq	0.2	11
Cult	2	
⑤ control HTPAN	32	30µl/Tube

④ HTPAN08 185bp + PD10

		55X
9114	1.2	66
2742	0.1	5.5
10xdNTP	3.2	176
10xPCR	3.2	176
H_2O	22.1	1215.5
FPAN Taq	0.2	11
Cult	2	
	32	30µl/Tube

⑤ Control HTPAN

⑥ Control PD10 Vector + LB Amp/Kan

Plate 3

⑤ HTPBHII + PD10

		24X
2752	1.2	28.8
2741	0.2	4.8
10xdNTP	3.2	76.8
10xPCR	3.2	76.8
H_2O	22	528
Taq	0.2	4.8
Cult.	2	
	32µl	30µl/Tube

⑥ control PD10 Vector + LB Amp Kan

⑦ Control HTPBHII

H7PBU1 + H7PAN08

1/17/95

plate 4

① H7PBY11 + PQE60		50X	② H7PAN08185 + PQE60		50X
28807	3	150	29107	0.2	10
28886	0.2	10	28805	0.2	10
10xdNTP	3.2	160	10xdNTP	3.2	160
10xPCR	3.2	160	10xPCR	3.2	160
H_2O	20.2	1010	H_2O	23	1150
Taq	0.2	10	Taq	0.2	10
Cult	2		Cult	2	
	32	30μl/tube		32	30μl/Tube

⊕ control H7PBY11

⊕ control ~~H7PBY~~ H7PAN08

⊖ control LB+Amp/Kan

PCR Prog 66.

95°C 5min
95°C 20sec
55°C 20sec } 30X
72°C 1min
72°C 7 1/2min
4°C hold

Run 10μl on gel with 1kb ladder.

Run by plate.

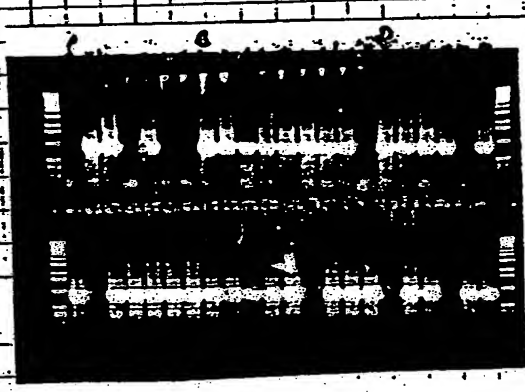
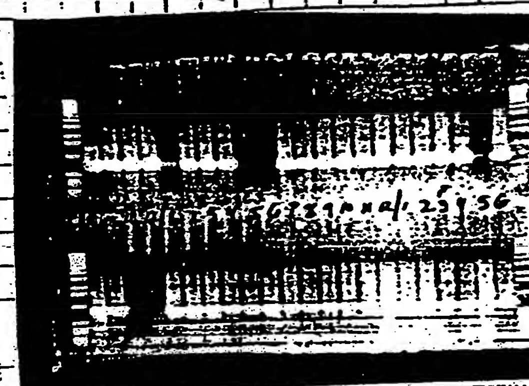
Plate 1 

Plate 2 

HTPB411 & HTPAN

11/17/95

Plate [1] + Plate [2]

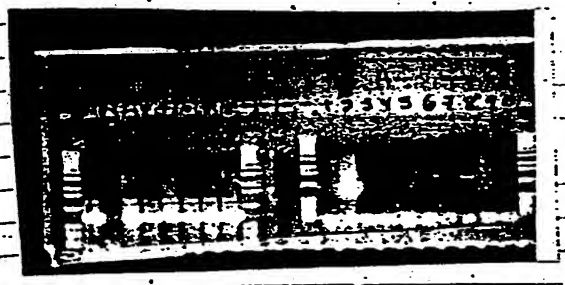


Plate [2]

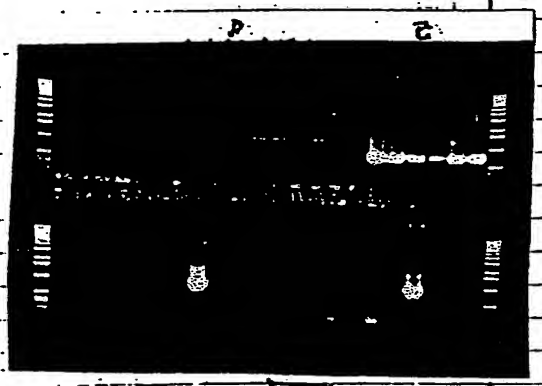


Plate [2]

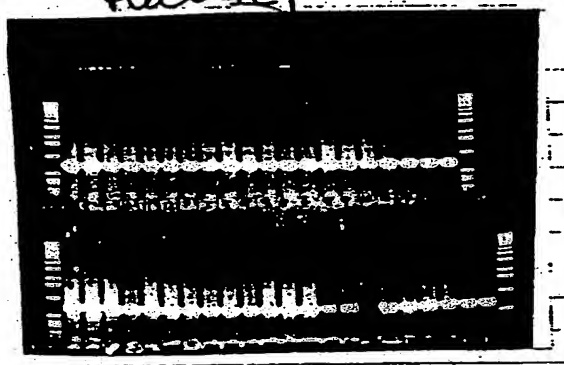


Plate [3]

Plate [4] Plate [4]

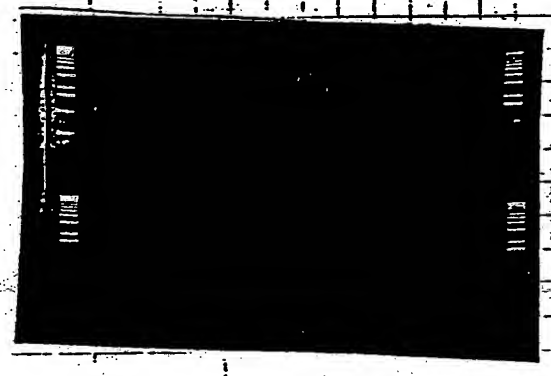
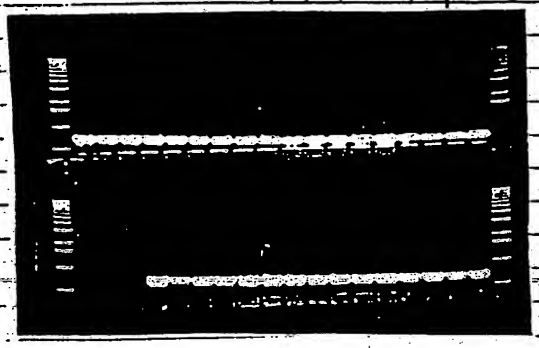


Plate [4]

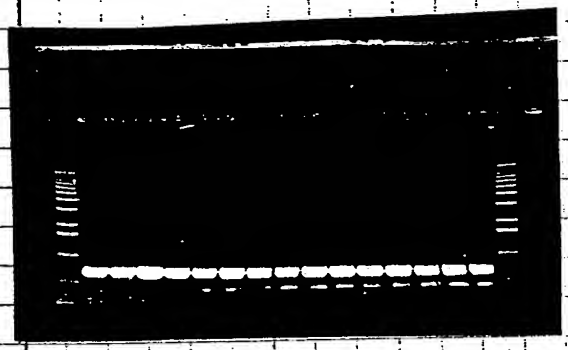


HTPAN08 ~ HTPB411

1/17/95

Plate 4

Controls



Controls

①	9111 + 2742	HTPAN08	5lbp PAZ
②	9112 + 2742	HTPAN08	185bp PAZ
③	9113 + 2742	↓	5lbp PD10
④	9114 + 2742	↓	185bp PD10
⑤	2752 + 2741	HTPB411	PD10
⑥	2887 + 2886	HTPB411	PQE660
⑦	2967 + 2865	HTPAN08	185bp PQE660

Inoculate 5ml LB + Kan/Amp
~~with 2 colonies ea~~
With 2 clones each of
HTPB411 + PQE660 - 4-1, 4-2, 4-3, 4-4 (A+B)
HTPAN08 185bp + PQE660: 4-1, 4-2, 4-3, 4-4 (A+B)

Inoculate 5ml TB + Amp
with 9 clones of each.
HTPAN08 5lbp + PAZ - 9
HTPAN08 185bp + PAZ - 9
HTPAN08 5lbp + PD10 - 3
HTPAN08 185bp + PD10 - 9
~~HTPB411 PQE660~~
HTPB411 PD10 - 9

Incubate 37°C O/N w/ Aeration

HTPB411 & HTPAN084

11/18/95

HTPB411 + PQE60.
HTPAN084 185bp + PQE60.

To 5ml LB + Amp/Kan add 200µl of ON Cultures.
Incubate 37°C w/ aeration till OD_{600} = 0.4 - 0.6. - 2hrs
Add 100mM IPTG to 2mM → 110µl.
Incubate 37°C 4hrs.
Spin 750µl of culture - induced and ① of uninduced of HTPAN084 HTPB411.
Remove supernatant
Resuspend pellet in 100µl H_2O
Add 100µl 2x dissociation Buffer.

2x dissociation Buffer:
- 0.125M Tris pH 6.9
- 4% SDS
- 20% Glycerol
- 10% β-Mercapto Ethanol
- 0.2% Bromophenol Blue

Mix well
Heat 100°C 5min
Spin 5min
Put on ice
Run 10µl on 12.5% Acrylamide stacking gel with Rainbow marker
Run at 150V in 1x Running Buffer for 1½ hrs. Tell dye front is at bottom of gel
Stain - ½ hr at 37°C
De Stain overnight at RT -

Boiling Mini preps.

HTPB411 & HTPAN08

1/18/95

HTPB411 + PAZ
HTPAN08 51bp PAZ
HTPAN08 185bp PAZ
HTPAN08 51bp PDID
HTPAN08 185bp PDID
HTPAN08 51bp PDE
} Boiling minis.

Some cultures did not grow
Spin 2ml cultures
Remove supernatant
Resuspend pellet in 750µl STET + RNase/Lysozyme.
Boil 1 min
Spin 10 min
Remove pellet
Add 750µl 13% PEG 8000 / 1.6M NaCl
Mix well
Spin 10 min
Remove supernatant
Wash pellet 1000µl 70% Ethanol
Spin 5 min
Remove supernatant
Allow pellet to dry at RT 30 min.
Resuspend pellet in 150µl TE
Run 2µl on gel with 1Kb ladder

A = HTPB411 + PAZ
B = HTPAN0851 + PAZ
C = HTPAN08185 + PAZ
D = HTPAN0851 + PDE
E = HTPAN08185 + PDID
F = HTPAN0851 + PDID

Set up digestions

HTPBYII & HTPAD08.

1/18/94.

for PAZ + PD10 Constructs
Digest: BamHI / XbaI

	5	40X
DNA	3	120
10X #2	21.6	874
H_2O	0.2	8
BamHI	0.2	8
XbaI	30µl	25µl/tube

For PCR Constructs
digest EcoRI / HindIII

	5	10X
DNA	3	30
HDX #2	21.6	216
H_2O	0.2	12
EcoRI	0.2	21
HindIII	30µl	25/tube

Incubate all reactions at 37°C O/N.

1/19/95.

Digestions of Mini Preps:

Run 10µl of Digestion on 1% TAE Agarose gel with 1Kb ladder

A HTPBYII + PAZ
B HTPAD08 51bp + PAZ
C 185bp + PAZ
D 51bp + PD10
E 185bp + PD10
F 51bp + PDEGD

Looks like some digested correctly

Clean-up and send in to be sequenced with internal primers.

HTPAN08 / HTPB411

1/19/95

Dilute HTPB411 + PA2 1,2,3,4
HTPAN08 516p + PA2 1,2,3,4
HTPAN08 1856p + PA2 1,3,4,5.

1:200 - use 10ul to transform into DH5α.

Thaw DH5α Chemically Competent cells on ice
To 100ul of thawed cells Add 10ul of Diluted DNA.
Incubate on ice 1hr
Heat 42°C 45sec
place on ice
Add 400ul LB
Incubate 37°C 1hr.
Plate 300ul onto 150mm LB+Amp plates.
Incubate 37°C O/N.

PCR HTPB411 + PA2
HTPAN08 516p + PA2
HTPAN08 985p + PA2
With a PA2 specific T7 primer and the 3' end primer

HTPAN		HTPB411	
T7	0.2	T7	0.2
2742	0.2	2741	0.2
10X	10	10X	10
10X	10	10X	10
H_2O	78.2	H_2O	78.2
Taq	0.4	Taq	0.4
DNA	1 ul of Diluted	DNA	1 of diluted
	100ul		100ul

4 Tubes of each

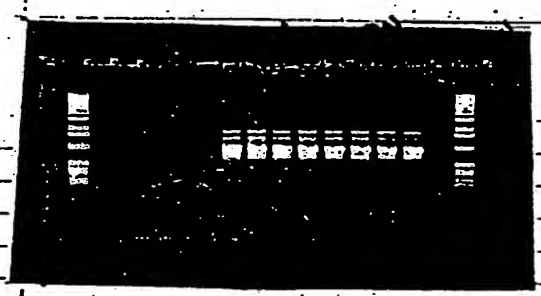
HTPAN08 / HTPB411

1/19/95

Run Program #66.

95°C	5min	
95°C	30sec	30x
55°C	20sec	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold.	

Run 5ul of PCR on 1% TAE gel with 1Kb ladder.



HTPB411 Did Not PCR - Need to get More P Clones.

HTPAN08 looks good.

HTPAN08 - precipitate with equal Vol 13% PEG / 1.6M NaCl
Mix Well
Spin 10min
Pour off Supernatant
Wash pellet 1ml 70% Ethanol
Spin 5min
Pour off supernatant
Allow pellet to dry at RT 15min.
Resuspend in 100 µl TE
Run 2ul on 1% gel with 1Kb Ladder

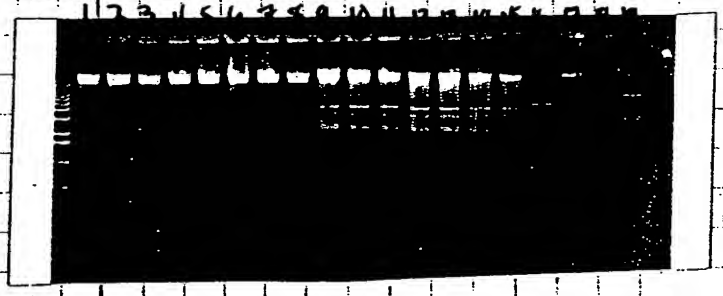
Don't know about upper band ~2Kb - but band looks good
Do TNT 1/20.

140 HTPAN08 / HTPD911

1/19/95

Clean up Boiling preps to submit for sequencing

Add TE to QS all samples to 400ul.
2x Phenol / SEVAG (1:1)
Extract with Equal Volume
2x SEVAG Extract with Equal Volume
Add 1/10 Vol (40ul) 3M NaOAc pH 5.3
2 Vol (~900ul) 100% Ethanol
Mix well
Set on ice 10 min
Spin 15 min
Remove Supernatant
Wash pellet 1000ul 70% Ethanol
Spin 5 min
Remove supernatant
allow pellet to dry at RT 5 min.
Resuspend pellet in 100ul TE.
Run Sulfon gel with 1kb ladder



(A) 1-4 • HTPAN08 518bp + PAZ
(B) 5-8 • HTPAN08 185bp + PAZ
(C) 9-12 HTPAN08 518bp + PDJO
(D) 13-16 HTPAN08 185bp + PDJO
(E) 17-19 - HTPAN08 518bp + POEC

Submit for Sequencing FP16 + RPO6.

IRIS Names:
(A) FAS518P1PAZRPO6/F
(B) FAS185B1PAZRPO6/RP1
(C) FAS518P1PDJRPO6/FP1
(D) FAS185B1PDJRPO6/FP1
(E) FAS518P1POERPO6/FP16

HTPAN08/HTPBH11

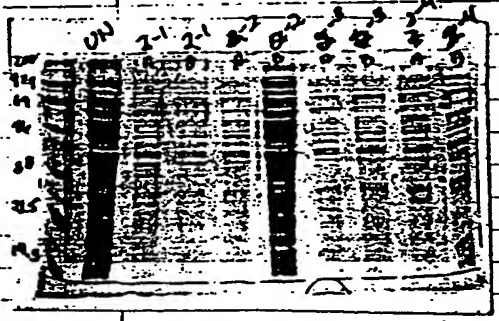
1/29/95

For
Inoculate 5ml LB+Amp/Kan
with HTPBH11+pQE60
HTPAN08 185 +pQE Constructs

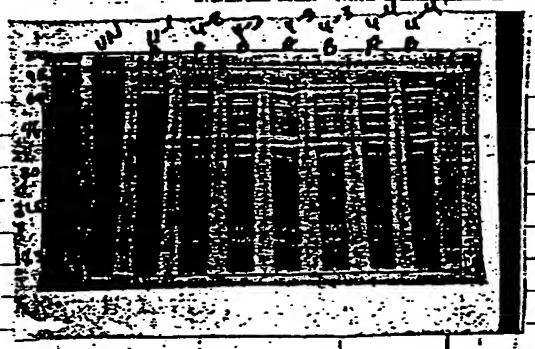
pg 135 -

Destained gel

HTPBH11+pQE60



HTPAN08 185bp +pQE60



Res more of Sample on gel - 10%.

Stain - Destain

Looks like #1 produced

1/29/95

Can't really tell. Try inducing more

142 HCPB411+HTPB4105

1/20/95 DO TNT with PCR'd T7 fragments (pg 139)

Rabbit Reticulocyte Lysate	12.5
T7 Buffer	1
T7 Polymerase	0.5
Amino Acid -Met	0.5
^{35}S methionine	2
RNasin	0.5
PCR Product	4
H_2O	4
	25 ul

Incubate 30°C 2 hours
Heat 90°C 5 min
Quick Spin
To 20ul of 2X dissociation buffer
Add 5ul of TNT

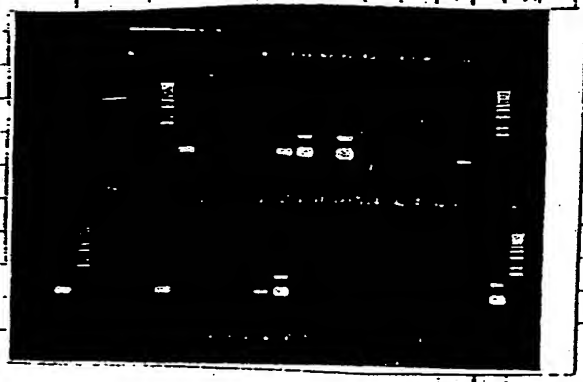
Run 15ul on 15% Acrylamide gel with C14 layed Rainbow marker
Run gel 150V 1½ hrs
until Dye front is almost to bottom.
Cut off stacking gel
Fix gel in 1 Fixative
10% HoAc; 30% MeOH
for 20 min at 37°C
Pour off fix
Amplify in 50 ml Amplify
for 20 min at 37°C
Dry gel 1½ hrs at 80°C.
Place on cellophane
Leave at RT over weekend

1/22/95 Inoculate 15ml TBT Amp / TBT Amp Kan 37°C O/N with cultures 1 (pg 149)

IL-6 PQE66 / PD10

pg 70

1/19/95



IL-6 PD10

Looks like Correct Clones

IL-6 PQE				IL-6 PD10			
1-A2	6 B10	11 D12	16 G5	1-A2	6-C4	11-E5	16 G1
2-A3	7 C1	12 E5	17 G8	2-A7	7-C5	12 E11	17 H2
3-A7	8 C4	13 E8	18 H5	3-B1	8-C8	13 F3	18 H3
4-A10	9 C9	14 E11	19 H8	4-B4	9-C9	14 F9	19 H5
5-A11	10 D10	15 F1	20 F10 ?	5-B11	10-D7	15 G8	20 H7

1/23/95

Inoculate 200ml LB + Amp/Kan with Cultures 1-20 of each. Incubate o/N at 37°C w/ aeration to Do Mini inductions

1/24/95

To 200 µl of fresh LB + Amp/Kan add 50 µl of fresh Cultures

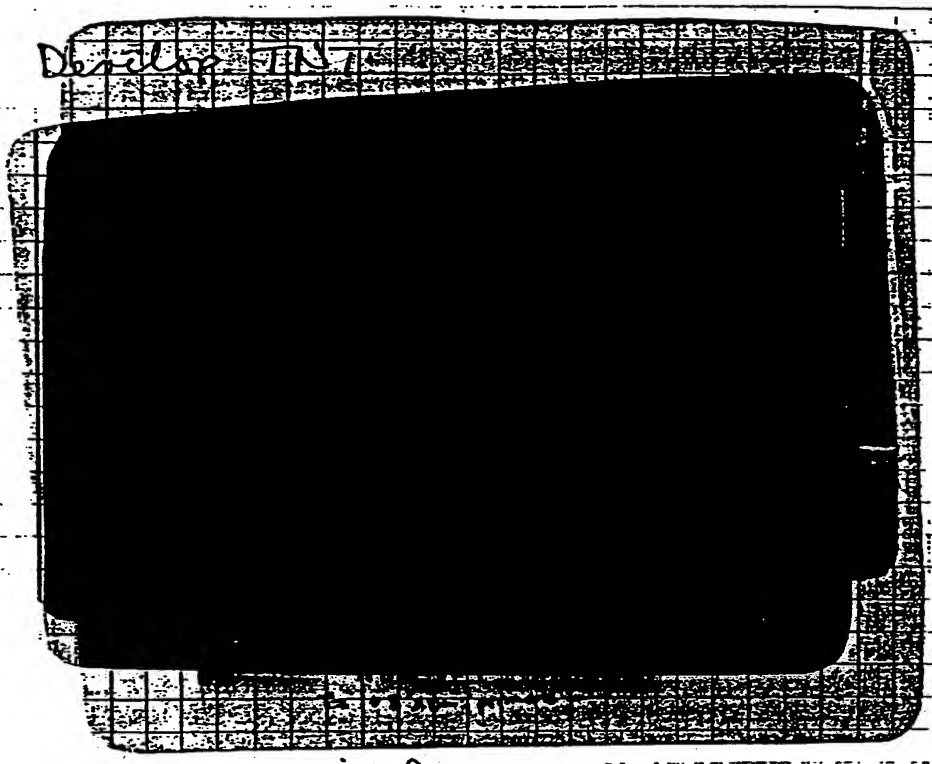
HTPB411 + HTPAN08

149

Pg 42

1/23/95

Develop INT



Qiagen Maxi Preps of:

HMEAA88
HTPAN08504 518bp + PA2 #1
+ PA2 #3
+ PA2 #4
185bp + PA2 #1
+ PA2 #3
+ PA2 #4
+ PA2 #5
+ PQE60 4-1
+ PQE60 4-2

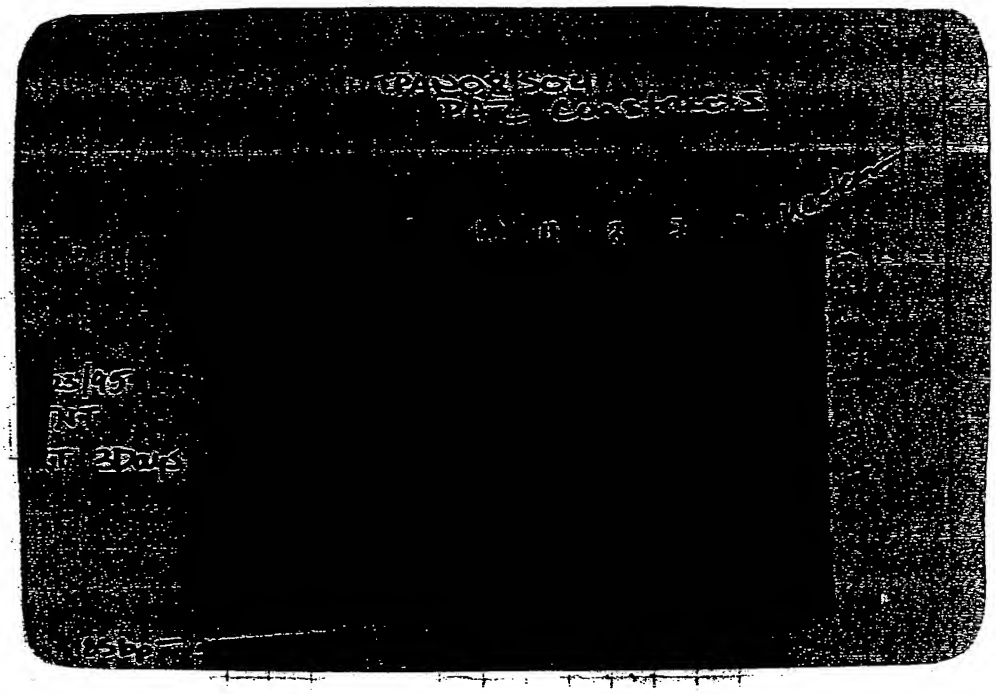
HTPB411515 + PA2 1

Make Glycerol stocks of all
800ul Bacterial Stock + 800ul 30% Glycerol

HTPB411 + HTPAN08

Py42

Develop TNT.



Qiagen Maxi Preps of:

HMEAA88
HTPAN08S04 51bp + PA2 #1
+ PA2 #3
+ PA2 #4
185bp + PA2 #1
+ PA2 #3
+ PA2 #4
+ PA2 #5
+ PQE60 4-1
+ PQE60 4-2

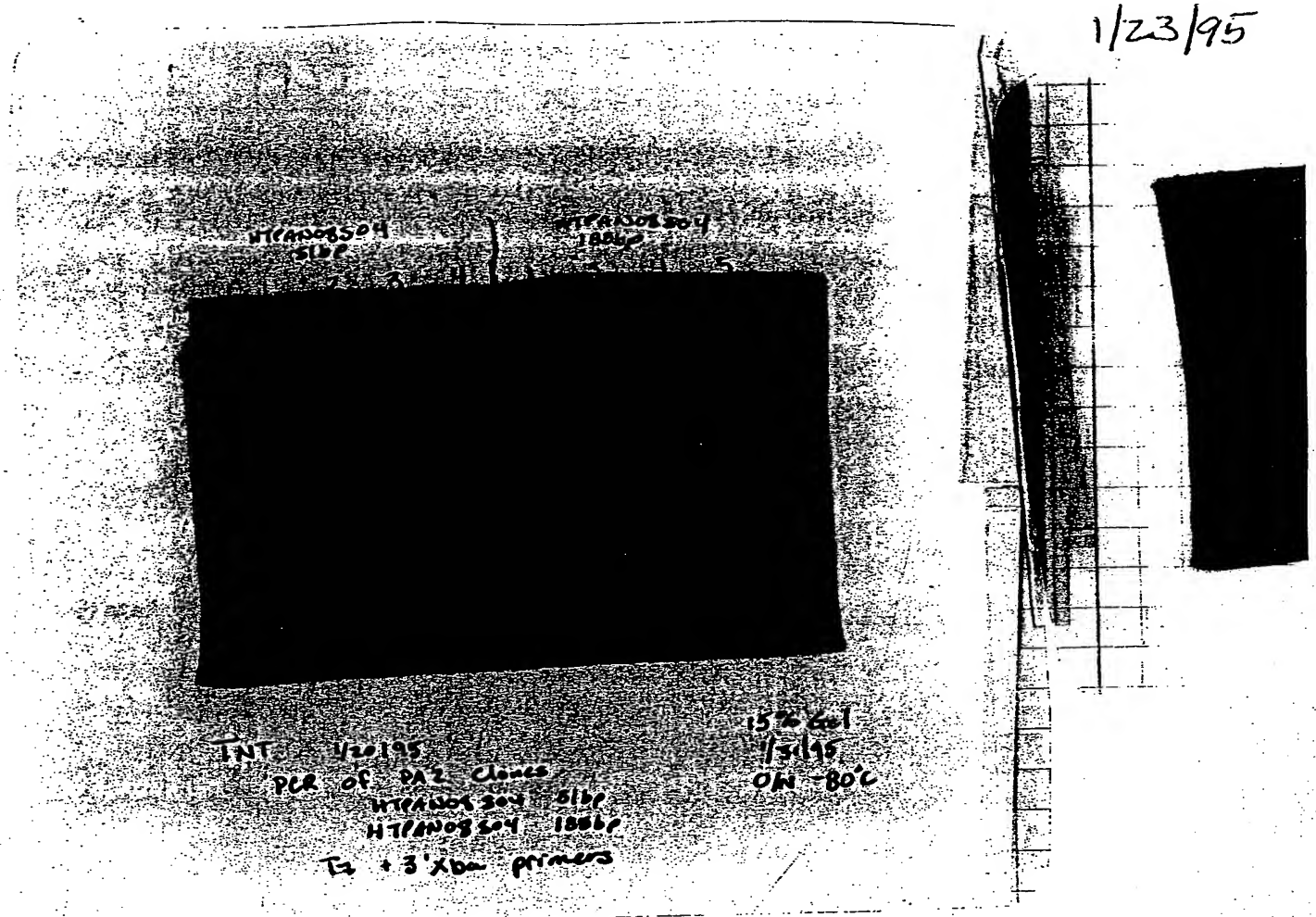
HTPB411S15 + PA2 1

Make Glycerol stocks of all
800ul Bacterial Stock + 800ul 30% Glycerol

HTPB411 + HTPAN08

pg42

1/23/95



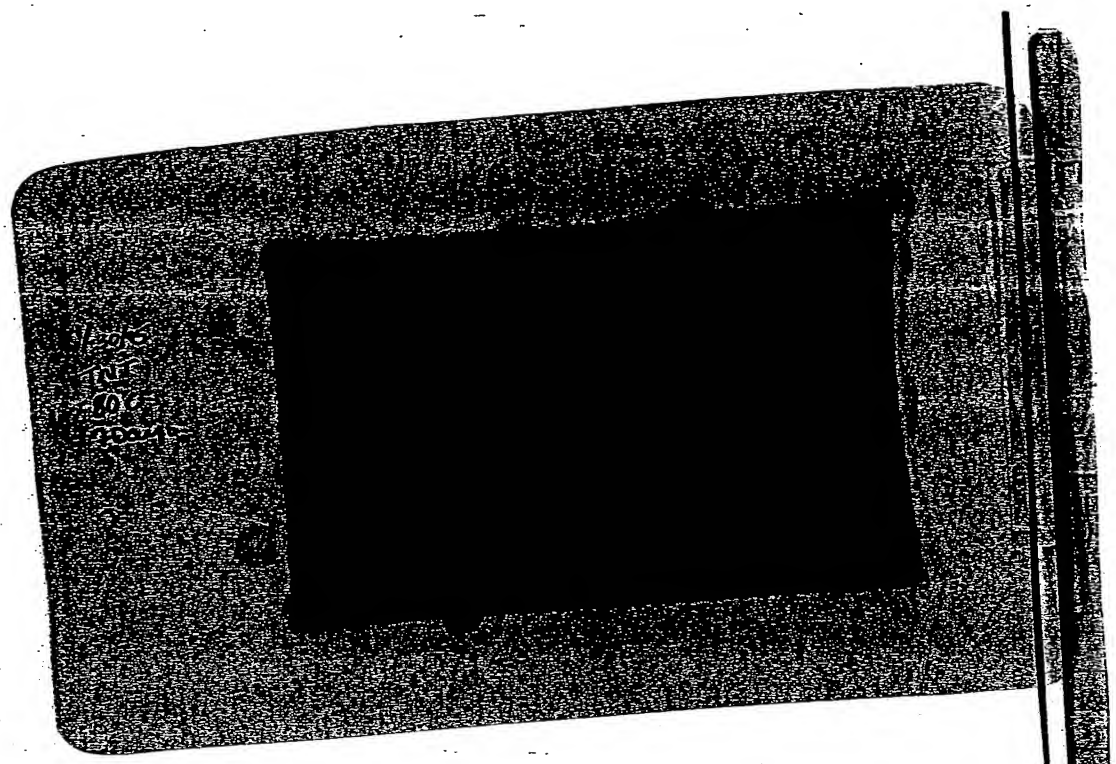
+PQE60 4-2
+PQE60

HTPB411515 + PA2 1

HTPBY11 + HTPAN08

Pg42

1/23/95



+PAE60 4-2

HTPBY11S15 + PAZ 1

Make Glycerol stocks of all

800ul Bacterial Stock + 800ul 30% Glycerol

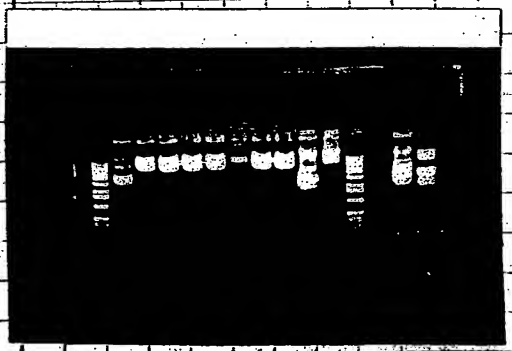
150

HIPAB08 7HRB4

1/23/95

Spin Culture 4.5 K 20 min
Pour off Supernatant
Resuspend pellet in 10 ml P1 + RNase
Let sit RT 5 min
Add 10 ml P2 - mix by scorling
~~Add~~ (added for cell Lysis) ✓
Add 10 ml P3 -
Incubate on ice 30 min
Spin 8K 30 min
Prepare Column
apply 10 ml QBT
allow to flow through
Apply Supernatant to Column through
~~to~~ KDa wipe -
Allow to flow through
Wash Column 30 ml Wash Buffer
Elute DNA 15 ml QF
Add Isopropanol 0.7 vol (10.5 ml)
Mix well
Spin 8K 30 min
Pour off Supernatant
Wash Pellet 10 ml 70% Etoh and (-20°C)
Spin 8K 15 min
Pour off Supernatant
Allow pellet to dry at RT for 10 min.
Resuspend pellet in 400 ul TE pH 7.6.

Run 1ul on gel with 1Kb Ladder
PA2 + PAEco



looks good

HTPAN08 / HTPB411

1/23/9

Read OD260/280.
Dilute 1:200 in H_2O

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm		
1 [illegible]	0.0466	0.0293	0.0014	1.6230	0.6162	0.47 ug/ml	188 ug
2 HTPAN08 PAZ 1	0.1271	0.0721	-0.0021	1.7411	0.5743	1.3 ug/ml	520 ug
3 HTPAN08 PAZ 2	0.1186	0.0663	-0.0002	1.7859	0.5599	1.2 ug/ml	480 ug
4 HTPAN08 PAZ 4	0.0983	0.0547	-0.0023	1.7855	0.5664	1.0 ug/ml	400 ug
5 HTPAN08 PAZ 8	0.0919	0.0533	-0.0003	1.7201	0.5814	0.92 ug/ml	368 ug
6 " 3	0.0033	0.0001	-0.0053	1.5858	0.6306	X	
7 " 4	0.1070	0.0630	-0.0008	1.6905	0.5915	1.1 ug/ml	440 ug
8 5	0.1304	0.0771	0.0035	1.7244	0.5799	1.3 ug/ml	520 ug
9 HTPAN08 PAZ 4-1	0.0967	0.0605	0.0045	1.6471	0.6071	0.97 ug/ml	388 ug
10 HTPAN08 PAZ 8-1	0.0481	0.0287	0.0002	1.6798	0.5953	0.48 ug/ml	192 ug
11 HTPAN08 PAZ 16	0.0502	0.0281	-0.0017	1.7434	0.5736	0.50 ug/ml	200 ug

400 ml

Dilute DNA to 250 ng/ml.
Submitt for determination
for HTPAN08 - FR16 & RP06
for HMEA88 RP01, RP02, RP04, Rev & For
HTPB411

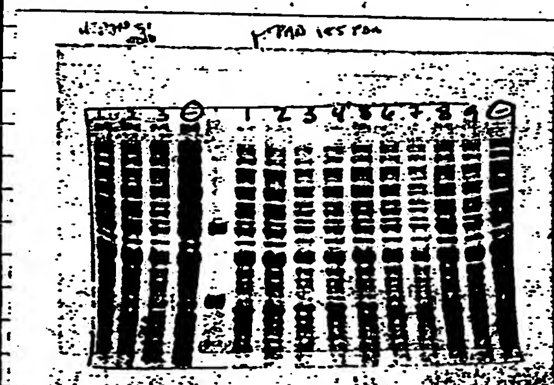
Inoculate 300 ml LB+Amp+Kan
with 5 ml HTPAN08 SO4 185 bp + PBSED
O/N culture. (L4-1)
Incubate at 37°C W/aeration
until OD600 ≈ 0.4-0.6
Add IPTG to 2 mM (1 eml of 100 mM IPTG)
Incubate at 37°C w/aeration
5 hours.
Spin cultures 8K 20 min
Resuspend in 30 ml 50 mM Gn HCl pH 8
Store 4°C till tomorrow

Pg 2 Book #9

1/24/95

1/2 Inoculate 200 ul LB + Amp Kan
with ⊕ clones of #
HTPAN08 516bp + PQ10 - 3
HTPAN08 185bp + PQ10 - 15 } 50 ul
HTPAN08 516bp + PQE60 - 17
Incubate w/ aeration 1 hour - 37°C
Add 100mM IPTG to 2mM → 25ul
Incubate 37°C w/ aeration 5 hrs.
HTPAN08 516bp + PQE60 - None Grew

Spin cultures
Add 20ul H2O to Resuspend pellet
Add 20ul 2X Dissociation Buffer
Heat 100°C 5 min
Run on 10% Acrylamide gel with
Low Mol. Weight Marker.
150 V 1 1/2 hrs.
Stain 30 min
DE Stain 30 min



Not enough gels to run remaining samples, but does not look like anything induced.

looks like from Sequence, there is a problem with 3'Xba end -
Remake primer

pg 9 book #27